EFFECTS OF BRASSINOSTEROIDS ON BORON AND SALT INDUCED CHANGES IN INDIAN MUSTARD *(BRASSICA JUNCEA* L.*)*

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

BOTANY

PRIYANKA VARSHNEY

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH – 202 002 (INDIA)

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Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those who are very close to our heart. I would like to dedicate my thesis to my grandparents, my parents and my husband

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Date: 04.05.2016

Priyanka.

(Signature of the Candidate)

Priyanka Varshney

(Name of the Candidate)

Certificate from the Supervisor

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Signature of the Supervisor:

Name and Designation: Dr. Qazi Fariduddin
Associate Prof

Department: Botany

(Signature of Chairman of the Department with seal)

Chairman
Department' of Botany
Aligarh Muslim University
ALIGARH-202002 (INDIA)

CHAIRMAN
DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH-202002 (INDIA)

Dated 04-05-2016

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This is to certify that **Mrs. Priyanka Varshney** Department of Botany has satisfactorily completed the course work/comprehensive examination and pre-submission seminar requirement which is part of her Ph.D. programme.

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Dr. QAZI FARIDUDDIN
BOYCAST Fellow (Germany)
Raman Fellow (USA)
Associate Professor

Plant Physiology Section
Department of Botany
Aligarh Muslim University
Aligarh-202002, U.P., India
E-mail: qazi_farid@yahoo.co
Phone: +91-9412172134

CERTIFICATE

Dated. May 04, 201

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(Qazi Fariduddin)
Research Supervisor

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Priyanka Varshney

List of abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
B	Boron
BAK1	BRI1-ASSOCIATED RECEPTOR KINASE1
BES1	BRI1-EMS SUPPRESSOR1
BIN2	BRASSINOSTEROID INSENSITIVE2
BKI1	BRI1 KINASE INHIBITOR1
BL	Brassinolide
BR(s)	Brassinosteroid(s)
BRI1	BRASSINOSTEROID RESPONSE INSENSITIVE1
BSK1	BR SIGNALING KINASE1
BSU1	BRI1 SUPPRESSOR1
BZR1	BRASSINAZOLE RESISTAT1
BZR2	BRASSINAZOLE RESISTAT2
CA	Carbonic anhydrase
Ca	Calcium
CAT	Catalase
Cd	Cadmium
C_i	Internal CO_2 concentration
Cl	Chloride
CN	Campestanol
CO_2	Carbon dioxide
CR	Campesterol
CS	Castasterone
DAS	Days after sowing
DDW	Double distilled water
DNA	Deoxyribonucleic acid
dSm^{-1}	Decisiemens per meter
E	Transpiration rate
EBL	24-epibrassinolide
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and agriculture organization
Fv/Fm	Maximum quantum yield of PSII
GPX	Glutathione peroxidase
GR	Glutathione reductase
g_s	Stomatal conductance
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
HBL	28-homobrassinolide

HCl	Hydrogen chloride
LSD	Least significant difference
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NBT	Nitrobluetetrazolium
NED-HCl	N-1-naphthyl-ethylendiamin hydrochloride
Ni	Nickel
NO_2^-	Nitrite ion
NO_3^-	Nitrate ion
NR	Nitrate reductase
O_2	Oxygen
$O_2^{\cdot -}$	Superoxide radical
$OH^{\cdot -}$	Hydroxyl radical
P	Phosphorous
PGRs	Plant growth regulators
P_N	Net photosynthetic rate
POX	Peroxidase
PSII	Photosystem II
RNA	Ribonucleic acid
$RO^{\cdot -}$	Alkoxy radical
ROS	Reactive oxygen species
Rubisco	Ribulose-1,5 biphosphate carboxylase/oxygenase
SOD	Superoxide dismutase
SPAD	Soil and plant analyzer development
TBARS	Thiobarbituric acid
XTHs	Xyloglucan endotransglucosylase/hydrolase
Zn	Zinc

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Chapter 1

INTRODUCTION

Chapter 1

INTRODUCTION

Brassica juncea L. Czern & Coss also known as Indian mustard or mustard greens or leaf mustard is a member of the family Brassicaceae. The primary center of origin of Indian mustard is thought to be central Asia while secondary centers are central and western China. It is chiefly grown in India, China, Pakistan, Canada, Germany, France, Australia and Poland. *Brassica* is a cool-season vegetable and prefers annual temperature of 6 to 27°C with an average temperature of 20°C, precipitation of about 350-550 mm and pH of 4.3 to 8.3. The plant is erect green annual herb attaining a height of one to two meter. It is an important seed crop grown for its oil content and used in edible and medicinal purposes. It is reported to be used as anodyne, apertif, emetic, diuretic, rubefacient, and stimulant. It is also a folk remedy for arthritis, footache and rheumatism (Mishra *et al.,* 2012). The leaves, stem and seeds of mustard are edible and a rich source of vitamin A and vitamin K. Seeds of *B. juncea* contain 25-30% fatty non-drying oil and glycoside sinigrine. Mustard oil is beneficial to human health because of its low content of saturated fats, ideal ratio of omega-3 and omega-6 fatty acids, and content of antioxidants, such as vitamin E. The leaves of young plants are also used as green vegetable as they supply enough sulphur and minerals in the diet. Mustard also has antioxidant activity and pharmacological effects on cardiovascular disease, cancer and diabetes. Recently, *B. juncea* has been explored for its biodiesel potential (Jham *et al.,* 2009).

India is the fourth largest oilseed economy in the world. Among the edible oilseeds cultivated in India, rapeseed-mustard contributes 28.6% in the total oilseeds production and ranks second after groundnut sharing 27.8% in the India's oilseed economy (Shekhawat *et al.,* 2012). However, production of rapeseed still remains insufficient to fulfill the daily requirements of the people (Khan *et al.,* 2002). The insufficient productivity can be attributed to various biotic and abiotic stresses like salinity, drought, chilling, heavy metals and nutrient stress (Allakhverdiev *et al.,* 2000; Sirhindi *et al.,* 2009; Fariduddin *et al.,* 2015; Varshney *et al.,* 2015).

Soil salinity has emerged as one of the serious problems that limit agricultural productivity as well as claiming substantial farmable area (Shahbaz and Ashraf, 2013). About 20% of the world's cultivated land area and 50% of all irrigated land are affected by salinity which results in a decline of the average yield of major crops, greater than 50% (Moud and Maghsoudi, 2008). Processes such as seed germination,

seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield (Cavusoglu *et al.,* 2007). High salt concentration primarily decreases the osmotic potential of soil solution creating water stress in plants. Secondly, it causes severe ion toxicity, since Na^+ is not readily sequestered into vacuoles, compared with halophytes. The excess amount of Na^+ ions in cells causes enzyme inhibition and metabolic dysfunction such as degradation of photosynthetic pigments (Chaves *et al.,* 2009). Salt stress causes decrease in the stomatal conductance (Parida *et al.,* 2004), internal CO_2 pressure and stomatal opening that affect gaseous exchange which results in the inhibition of photosynthesis in the salt affected plants (Iyenger and Reddy, 1996). This decrease in photosynthesis under saline conditions is considered as one of the most important factor responsible for reduced plant growth and productivity (Manikandan and Desingh, 2009). Moreover, salinity enhances the over-production of reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide in plant cells (Mahajan and Tuteja, 2005; Ahmad and Prasad, 2012) that triggers lipid peroxidation, DNA damage, inhibition of photosynthesis and disturbance in mineral nutrient status (Turan and Tripathy, 2012). All of these cause adverse pleiotropic effects in plants.

Nutritional (mineral) stress has increased at an accelerating pace in recent years due to supply of excess nutrients Boron (B) is an essential micronutrient required for normal growth and development of higher plants. Like many other elements, the concentrations of B in the soil that are above the tolerable limits lead to an abiotic stress factor that prevent plant growth and development resulting into reduced crop yield and quality (Nable *et al.,* 1997). The threshold concentration between deficiency and toxicity of B is very narrow (Nable *et al.,* 1997). B naturally occurs at toxic levels worldwide i.e., in many places where the soil is irrigated with B-rich water or in arid and semi-arid regions where, B is enriched as natural deposits due to poor drainage and geothermal activity. High levels of B in soil results in reduced cell division, root and shoot growth, stomatal conductance (Lovatt and Bates, 1984; Nable *et al.,* 1990), leaf chlorophyll and CO_2 assimilation (Papadakis *et al.,* 2004; Reid, 2010; Guidi *et al.,* 2011; Landi *et al.,* 2013) whereas, it increased membrane leakiness, peroxidation of lipids and altered activities of antioxidant pathways (Karabal *et al.,* 2003; Keles *et al.,* 2004). B toxicity also impairs nitrogen assimilation pathways by affecting key enzymes (Herrera-Rodriguez *et al.,* 2010) and disrupts RNA splicing (Reid, 2007a).

Moreover, B interferes with transcription and/or translation mechanisms by binding to biological compounds containing two hydroxyl groups in cis-configuration such as ribose, ATP, NADH, NADPH (Reid, 2010).

Recently, co-occurrence of B and salinity in natural and agricultural environments has led to research reports on the interaction of these two stresses as they affect crop performance (Ben-Gal and Shani, 2002; Yermiyahu *et al.,* 2008). The interactive effects of salinity and B are interesting and provide insight on several levels. When both stresses occur together, studies have indicated that salinity may reduce or increase toxic effects of B (Yermiyahu *et al.,* 2008). These negative impacts can be additive (Grieve and Poss, 2000; Alpaslan and Gunes, 2001; Wimmer *et al.,* 2003) or antagonistic (Ferreyra *et al.,* 1997; Bastias *et al.,* 2010; Smith *et al.,* 2010), leading to an attenuation of the detrimental effect in plants, over a range of concentrations.

Brassinosteroids (BRs) have emerged as a new class of plant-specific steroidal hormones characterized by their polyhydroxylated sterol structure which are ubiquitously distributed throughout the plant kingdom (Bajguz and Piotrowska-Niczyporuk, 2014). The essential role of BRs in regulating wide array of physiological and molecular responses in plants was established when mutants deficient in either BR biosynthesis or perception were identified (Clouse *et al.,* 1996; Li and Chory, 1997). BRs are involved in a number of physiological processes, like cell elongation and cell division in stem, reproductive and vascular development, membrane polarization, proton pumping, and abscission of plant organs (Yang *et al.,* 2011). Brassinosteroids also influence various other developmental processes in plants like seed germination, rhizogenesis, flowering and induced synthesis of nucleic acids and of proteins (Khripach *et al.,* 2003), activation of several enzymes and photosynthesis (Fariduddin *et al.,* 2014a), and increased fruit set (Kamuro and Takatsuto, 1999; Ali *et al.,* 2006). In addition to this, BRs are also involved in conferring tolerance to a wide range of abiotic stresses like drought stress (Zhang *et al.,* 2008), flooding (Liang and liang, 2009), salinity stress (Shahid *et al.,* 2011; Fariduddin *et al.,* 2013), high and low temperature stress (Ogweno *et al.,* 2008; Jiang *et al.,* 2013), and heavy metal stress (Anuradha and Rao, 2009; Arora *et al.,* 2012).

B. juncea is considered a moderately salt tolerant crop (Ashraf and McNeilly, 2004). However, its adaptability to low rainfall areas, where B and salinity occur simultaneously is poorly understood (Burton *et al.,* 2004). The hypothesis tested is

that the application of BRs will ameliorate the stress generated by B and salinity on the growth, photosynthesis, antioxidant system and economical yield of test plants. *Brassica juncea* L. Czern & Coss is selected as a test crop due to its wide consumption throughout the world and acceptability by the local farmers as a cash crop.

The following objectives were envisaged while planning the experiments:

- To investigate the sensitivity of two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* to varied concentrations of boron.
- To explore the response of *Brassica* varieties to varied levels of NaCl, in the presence or absence of boron.
- To dissect the effects of two exogenously sourced analogues (28-homobrassinolide and 24-epibrassinolide) of brassinosteroids under different levels of boron in these two varieties of mustard.
- To assess the ameliorative role of best suited brassinosteroid analogue in mustard plants, under the impact of NaCl and boron together.
- To establish the relative responses of mustard to varied levels of NaCl and B.
- To select the growth, physiological or biochemical traits, showing maximum response to the treatment that may be designated as marker to forecast the growth pattern and crop productivity, and to ensure corrective measures.

Chapter 2

Review Of Literature

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REVIEW OF LITERATURE

2.1 SALT STRESS

The environmental stress is a major area of scientific concern because it constraints plant growth as well as crop productivity (Shanker and Venkateswarlu, 2011). Plants are frequently exposed to various environmental factors including salinity, drought, heavy metal toxicities and temperature extremes impairing crop production worldwide (Mahajan and Tuteja, 2005). Out of them, salinity is one of the most destructive abiotic stresses, limiting crop production in arid and semi-arid areas, where soil salt content is naturally high and precipitation may be insufficient for leaching (Zhao *et al.*, 2007). Over 6% of the world's land is affected by salinity and out of the current 230 million hectares of irrigated land, 45 million hectares (19.5%) is salt-affected (FAO, 2008). In addition, the increased salinity of arable land is expected to have devastating global effects, resulting in up to 50% land loss by the middle of the twenty-first century (Mahajan and Tuteja, 2005). Soil salinity can be defined as the concentration of dissolved mineral salts in soil solution as a unit of volume or mass basis (Ghassemi *et al.*, 1995) whereas, the sodicity expresses the presence of sodium, attached (exchangeable) to the surface of the soil matrix. Sodic soils contain excessive concentrations of exchangeable sodium (Bernstein, 1975). Sodicity differs from salinity by being specific to only one salt (sodium) rather than a range of salts and it is a measure of ions on clay surfaces rather than in the soil solution. Soil salinity occurs naturally in marshes or areas in which salt is already a part of the soil composition. This is called primary salinization, and plants that grow here are adapted to the soil composition. Secondary salinization occurs when soils that once had a low concentration of salt become rich in salt because of irrigation and poor drainage (Zhu, 2007). The plants that grow on these soils are not adapted to tolerate salty conditions, thus, when soils become more saline, they struggle to thrive. Salt stress normally affects all the major processes such as germination, growth, photosynthesis, water relation, nutrient imbalance, oxidative stress, and yield. These salt-induced changes in plant cells due to: (1) disruption of ionic equilibrium is influx of Na^+ dissipates the membrane potential and facilitates the uptake of Cl^- down the chemical gradient. (2) Na^+ is toxic to cell metabolism and has deleterious effect on the

functioning of some of the enzymes. (3) higher concentration of Na^+ causes osmotic imbalance, membrane disorganization, reduction in growth, inhibition of cell division and expansion. (4) high Na^+ level also leads to the reduction in photosynthetic rate and production of ROS (Yeo, 1998). However, plants have developed various morphological, physiological and biochemical strategies to overcome the salt stress. Several mechanisms work in a coordinated manner in order to minimize the damage caused by salinity.

2.1.1 Effect of salt stress on seed germination

Seed germination is one of the most fundamental and vital phases in the growth cycle of a plant that determines the final yield. However, it has been established that salinity adversely affects the process of seed germination in various plants like *Posidonia* (Fernandez-Torquemada and Sanchez-Lizaso, 2013), *Zea mays* (Khodarahmpour *et al.,* 2012), *Oryza sativa* (Xu *et al.,* 2011), and *Brassica* spp. (Ibrar *et al.,* 2003; Ulfat *et al.,* 2007). Salinity affects the process of seed germination by affecting imbibition of water due to lower osmotic potential of germination media (Khan and Weber, 2008), causes toxicity which changes the activity of enzymes of nucleic acid metabolism (Gomes-Filho *et al.,* 2008), alters protein metabolism (Dantas *et al.,* 2007), disturbs hormonal balance (Khan and Rizvi, 1994), and reduces the utilization of seed reserves (Othman *et al.,* 2006). Therefore, the germination rate and percentage of germinated seeds, at a particular time vary considerably among species and cultivars. Kaveh *et al.* (2011) established a negative correlation between salinity and the rate of germination percentage in *Solanum lycopersicum.*

2.1.2 Effect of salt stress on plant growth

One of the initial effects of salt stress is the reduction in growth rate (Ghoulam *et al.,* 2002). Presence of salts in the soil water may inhibit plant growth for two reasons Firstly, it reduces the ability of the plant to take up water and this leads to a reduction in the growth rate. This is referred to as the osmotic or water-deficit effect of salinity. Secondly, if an excessive amount of salts enters the plant transpiration stream this could develop injury to cells in the leaves and cause further reductions in growth (Greenway and Munns, 1980). These salinity effects have threefold effects viz. reduce water potential and cause ion imbalance or disturbances in ion homeostasis and toxicity; this altered water status leads to initial growth reduction and limitation of plant productivity. The detrimental effects are observed both at cellular and the whole

plant level. Salinity induced reduction in vegetative and reproductive development has profound implications where shoot growth is affected more than that of root (Lauchli and Epstein, 1990). The effects of salt stress on plant growth are summarised in table 1.

Table 1: Effect of salt stress on plant growth in various species

Crop/ plant species	Effect	References
Foeniculum vulgare	Height, fresh and dry mass, and yield were significantly affected by salinities developed due to irrigation water	Semiz *et al.*, 2012
Morus alba	Decreased plant growth subjected to salt stress	Ahmad and Sharma, 2010
Suaeda salsa	Plant height, number of branches, length of branches, and diameter of shoot were significantly affected by salt stress	Guan *et al.*, 2011
Helianthus annuus	Significant reduction in plant growth	Akram and Ashraf, 2011
Panicum miliaceum	Reduction in plant growth	Sabir *et al.*, 2011
Lycopersicon esculentum	Significant decrease in growth and photosynthetic attributes	Fariduddin *et al.*, 2012
Atriplex species	Salt stress decreased the leaf mass	Belkheiri and Mulas, 2013
Solanum tuberosum	Reduced the growth of stressed plants	Daneshmand *et al.*, 2010
Brassica juncea	Significantly decreased the growth	Hayat *et al.*, 2011a; Ahmad *et al.*, 2012
Lycopersicon esculentum	Growth of the plants was reduced subjected to saline stress	Tantaway *et al.*,2009
Triticum aestivum	Salt induced reduction in dry mass of shoots	Iqbal and Ashraf, 2013

Spartina alterniflora	Sharp reduction in survival rate, relative growth rate, rhizome number and leaf chlorophyll content	Li *et al.*, 2010
Juglans regia	Fresh and dry mass of shoot and root decreased with increasing salt stress	Akca and Samsunlu, 2012
Glycine max	Reduction in shoot and root mass, total biomass, plant height, and leaf number	Dolatabadian *et al.*, 2011
Populous alba	The growth of the plants was reduced	Imada and Tamai, 2009
Ocimum basilicum	Fresh mass decreased significantly with an increase in salinity level	Heidari, 2012
Brassica napus L.	Salt stress caused significant reduction in fresh and dry mass	Rasheed *et al.*, 2014
Cajanus cajan	Significant reduction in the growth of the plants	Amuthavalli and Sivasankaramoorthy, 2012

2.1.3 Effect of salt stress on plant water uptake

Water potential is an important physiological trait for determining the water status of the plants (Parida and Das, 2005). According to Romero-Aranda *et al.* (2001), an increase of salt in the root medium could lead to a decrease in leaf water potential and hence, affects many metabolic processes. However, at low or moderate salt concentration (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. In *Cucumis sativus*, it has been reported that the water potential decreased linearly with increasing salinity levels (Khan *et al.*, 2013). Moreover, leaf water potential also decreased, in response to salt stress in *Chenopodium quinoa* (Eisa *et al.*, 2012), *Shepherdia argentea* (Qin *et al.*, 2010) and *Iris lacteal* (Wen-Yuan *et al.*, 2012).

2.1.4 Effect of salt stress on photosynthesis and related attributes

The decrease in photosynthesis under saline conditions is considered as one of the most important factors restricting plant growth and productivity (Manikandam and Desingh, 2009). Photosynthesis is inhibited when higher concentrations of Na^+ and/or Cl^- are accumulated in the chloroplast and chlorophyll content is directly correlated to

the photosynthesis in plants (Zhang *et al.,* 2005). There are some other factors that reduce photosynthetic rate under salt stress including, dehydration of cell membranes which reduce their permeability to carbon dioxide, enhanced senescence, changes in enzyme activity induced by alterations in cytoplasmic structure, and negative feedback by reduced sink activity (Iyengar and Reddy, 1996). Salt toxicity could lead to the death of leaves which reduces the total photosynthetic leaf area and finally a decrease in the supply of photosynthates thereby affecting the overall carbon balance necessary to sustain growth and development (Munns, 2002). The overall reduction in net photosynthetic rate and its related attributes has been observed in various crops which is summarized in table 2.

Table 2: Effect of salt stress on various photosynthetic attributes in different plant species

Crop/ plant species	Effect	References
Cucumis sativus	Severe reduction of net photosynthetic rate	Shu *et al.,* 2012
Brassica juncea	Significantly decreased the net photosynthetic rate, transpiration rate and stomatal conductance	Ahmad *et al.,* 2012; Hayat *et al.,* 2011a
Triticum aestivum and *Hordeum Vulgare*	Increase in NaCl concentration in the nutrient solution resulted in the closure of stomata and decreased net CO_2 assimilation rate	Dulai *et al.,* 2011
Phaseolus vulgaris	Photosynthetic rate and stomatal conductance decreased gradually with salinity level in salt-sensitive species	Bayuelo-Jimenez *et al* 2012
Abelmoschus esculentus	Decreased photosynthetic rate and stomatal conductance	Shahid *et al.,* 2011
Brassica juncea	Decreased maximum quantum yield of PSII	Mittal *et al.,* 2012
Oryza sativa	Reduced the contents of chlorophyll a and b in leaves	Amirjani, 2011
Vigna radiata	Total chlorophyll as well as the intensity of	Saha *et al.,* 2010

	chlorophyll fluorescence decreased	
Oryza sativa	Reduction in chlorophyll a, chlorophyll b, and carotenoids contents	Chutipaijit *et al.*, 2011
Cucumis melo	Significant reduction in stomatal conductance	Kusvuran *et al.*, 2011
Chenopodium quinoa	Photosynthetic rate decreased under high salinity	Eisa *et al.*,2012
Phaseolus vulgaris	Photosynthetic rate and stomatal conductance decreased gradually with salinity in salt-sensitive species	Bayuelo-Jimenez *et al.*, 2012
Shepherdia argentea	Salinity induced photo-inhibition leads to lowest net photosynthetic rate, stomatal conductance and internal CO_2 concentration values	Qin *et al.*, 2010
Triticum aestivum	Photosynthetic rate, stomatal conductance, and transpiration rate decreased in response to NaCl	Sharma *et al.*, 2005; Hayat *et al.*, 2014
Jatropha curcas	Net carbon assimilation rate, stomatal conductance and transpiration rate decreased significantly	Campos *et al.*, 2012
Citrus	Reduction in net photosynthetic rate, stomatal conductance and quantum yield of PSII	Lopez-Climent *et al.*, 2008
Pisum sativum	Salt stress caused decrease in the stomatal conductance	Hernandez and Almansa, 2002
Atriplex portulacoides	Reduction in net photosynthetic rate was accompanied by lower stomatal conductance and internal CO_2 concentration values in response to salt stress	Redondo-Gomez *et al.*, 2007
Hordeum vulgare	Photosynthetic rate and quantum yield of photosystem II significantly decreased	Kalaji *et al.*, 2011

2.1.5 Salt stress and ionic toxicity

The presence of excessive soluble salts in the soil competes with the uptake and metabolism of mineral nutrients that are essential to plants. The appropriate ion ratio provides a tool to the physiological response of a plant in relation to its growth and development (Wang *et al.,* 2003). However, increased salt uptake induces specific ion toxicities like that of high Na^+, Cl^-, or SO_4^{2-} that decrease the uptake of essential nutrients like phosphorus (P), potassium (K^+), nitrogen (N), calcium (Ca^{2+}) and magnesium (Mg^{2+}) which results in the nutritional disorder that eventually leads to a decrease in biological yield (Grattan and Grieve, 1999). Higher NaCl concentration has been reported to increase the level of Na^+ and Cl^- ions and decrease in those of Ca^{2+}, K^+ and Mg^{2+} in various plants (Bayuelo-Jimenez *et al.,* 2003). Salinity enhances Na^+ content in *Vicia faba* while the ratio of Na^+/K^+ was decreased (Gadallah, 1999) thus, indicating a negative relationship between Na^+ and K^+. In addition, many of the deleterious effects of Na^+ seem to be related to the structural and functional integrity of membranes (Kurth *et al.,* 1986). Salinity stress causes an increase in the level of Na^+ and Cl^- in *Atriplex griffithii* in root, stem, as well as in the leaves, and the highest ion accumulation was found in leaves followed by stem and root suggesting a positive relationship between Na^+ and Cl^- concentration. The Ca^{2+} content was reduced in shoots and leaves of *A. griffithii* plants grown at high salinity however, being stable in roots, K^+ content was reduced with increased levels of salinity, particularly in leaves. Salinity stress decreased Ca^{2+} and Mg^{2+} content in the leaves of *Brugueira parviflora* which increased membrane stability but decreased chlorophyll content (Parida *et al.,* 2004).

2.1.6 Salt stress and antioxidant system in plants

Besides direct impact of salinity on plants, a common consequence of salinity is induction of excessive accumulation of reactive oxygen species (ROS) leading to oxidative stress in plants (Demiral and Turkan, 2005). These ROS are continuously generated during normal metabolic processes in mitochondria, peroxisomes and cytoplasm which disturb normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when produced in excess (Hernandez *et al.,* 2001; Ahmad *et al.,* 2010). It has been shown that the production of ROS during environmental stresses such as salinity is one of the main causes for the decrease in crop productivity (Demiral and Turkan, 2005). Therefore, regulation of ROS is a crucial process to

avoid unwanted cellular cytotoxicity and oxidative damage (Halliwell and Gutteridge, 1989). To overcome salt-mediated oxidative stress, plants detoxify ROS by upregulating antioxidative enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPX). A correlation between antioxidant capacity and salinity tolerance has been reported in several plant species such as *Oryza sativa* (Demiral and Turkan, 2005), *Beta vulgaris* (Bor *et al.,* 2003), *Helianthus annuus* (Noreen *et al.,* 2009), *Triticum aestivum* (Ashraf *et al.,* 2010), and *Carthamus tinctorius* (Siddiqi, 2010).

Proline accumulation is also believed to improve adaptation of plants to salt and drought stresses by scavenging free radicals and stabilizing membranes to maintain the proper conformation of proteins under stress conditions (Chen and Dickman, 2005). Proline is also reported to play a significant role in reducing the photo-damages of thylakoid membranes by scavenging the superoxide radicals (Ashraf and Foolad, 2007). Proline accumulation under dehydrated conditions is mainly due to increased biosynthesis and decreased degradation. Enhanced synthesis of proline under drought or salinity has been involved in the alleviation of stress in various plants such as *Cynodon dactylon* (Hameed and Ashraf, 2008), *Pisum sativum* (Noreen and Ashraf, 2009), *Brassica juncea* (Hayat *et al.,* 2011a), *Saccharum officinarum* (Chaum and Kirdmanee, 2009), *Panicum miliaceum* (Sabir *et al.,* 2011). The ROS regulatory pathways are very flexible and the degree of redundancy and cross talk between different branches of ROS network, as well as the way in which the network senses and the components through which ROS signals transmit need further investigation.

2.1.7 Salt stress and yield

All detrimental effects of salt stress on plants ultimately lead to reduction in crop yield that may be attributed to low production, expansion, senescence, and physiologically less active green foliage (Wahid *et al.,* 1997), therefore, reduced photosynthetic rate might be a supplementary effect (Seemann and Critchley, 1985). Different yield components of *Vigna radiata* were significantly affected by salinity stress as reported by Nahar and Hasanuzzaman (2009). Number of pods per plant, seeds per pod, and seed weight were negatively correlated with salinity levels. Moreover, in *F. vulgare*, plant growth parameters including plant height, fresh mass,

and yield were affected significantly by salinity stress resulting from over irrigation (Semiz *et al.*, 2012).

2.2 BORON

Most plants require a number of nutrient elements in order to successfully complete their life cycle. Boron (B) is one of the important essential micronutrient, required in relatively small quantities (less than 10 mmole kg^{-1} of dry mass) to maintain normal growth and development in plants.

2.2.1 Distribution of boron in environment

Boron is widely distributed in lithosphere and hydrosphere and its concentration varies from 5-10 mg kg^{-1} in rocks (Shorrocks, 1997), 3-30 µg kg^{-1} in rivers (Power and Woods, 1997) and 4-5 mg L^{-1} in ocean (Lemarchand *et al.*, 2000). Boron is never found in its elemental form in nature, but is found in rocks and concentrated in deposits as borates i.e. bound to oxygen together with sodium, calcium, silicon, or magnesium. At least 200 minerals are known to contain elemental B, however, only few (borax, ulexite, kernite, boric acid, colemanite) are of commercial importance (Parks and Edwards, 2005). In the environment, B is primarily driven from the weathering of minerals, containing this element (Kot, 2009). During rock weathering, B goes easily into soil solution mainly as boric acid (H_3BO_3) and is readily available for plant uptake (Nable *et al.*, 1997). Boron concentrations in soil vary from 2 to 200 mg B kg^{-1}, but generally less than 5-10% is available to plants (Diana, 2006). However, availability of B in soil can be affected by several factors such as its pH, texture, temperature and organic matter where soil pH being one of the most important parameters. Boric acid (H_3BO_3) is a very weak acid and remains in undissociated form below pH 7 but at alkaline pH, it dissociates to form borate anion. Therefore, in neutral or slightly acidic soils, B exists mainly in undissociated form (H_3BO_3), and this form is absorbed by plant roots which is mobile and easily gets leached under high rainfall conditions leading to B deficiency in plants. On the contrary, under low rainfall conditions, B cannot leach in ample quantity and, therefore may accumulate to levels that becomes toxic to plant growth (Reid, 2007a). Moreover, various anthropogenic sources could further increase the concentration of B in soil that is toxic for plants. The most important source of B is irrigation water (Nable *et al.*, 1997) but other includes waste from surface mining, fly ash, and industrial chemicals (Kot, 2009).

2.2.2 Boron transport in plants

During recent years, various molecular aspects involved in B uptake by root cell and its allocation in plants have been revealed which helped to understand the mechanism of B transport and utilization in plants. It may provide a novel strategy to improve crop plant for better nutrient transport properties that allows plants to withstand boron stress.

2.2.2.1 Boron uptake and distribution in plants

Boron occurs in soil solution predominantly as soluble boric acid (H_3BO_3) and plants take up B from soil in this form. The uptake of B by plant roots from the soil solution has long been believed that this nutrient is passively absorbed by the root cell through simple diffusion across lipid bilayer (Brown *et al.,* 2002; Tanaka and Fujiwara, 2007). However, recent physiological studies have revealed the involvement of channel-mediated facilitated diffusion and energy-dependent active transport against concentration gradients in transport system of B (Dannel *et al.,* 2001; Stangoulis *et al.,* 2001a, b). Recently, Takano *et al.* (2006) identified a novel boric acid channel (NIP5;1) in *A. thaliana*. NIP5;1, a protein similar to aquaporin acts as a transporter required for efficient uptake of B which is localized and expressed in plasmalemma of root epidermal, cortical and endodermal cells. In rice, NIP3;1 shows a close homolog sequence to NIP5;1 and it has also been identified as a boric acid channel required for the uptake of B into the root cells under B-limiting conditions but, unlike NIP5;1, it has also been detected in shoot (Hanaoka and Fujiwara, 2007).The first B transporter required for efficient xylem loading was identified as BOR1 in *A. thaliana*, which is accumulated in plasma membrane of pericycle cells under low levels of B (Takano *et al.,* 2002). *Arabidopsis* and rice have seven and four BOR1 or BOR1-like genes, all likely to encode efflux transporters, with different physiological functions and location within the cell. Expression of BOR1 and NIP5;1 is both upregulated under B deficient conditions but with different mechanisms. In addition, BOR4 (a paralog of BOR1) is an active efflux transporter of B and is localized in the outer side of the epidermal plasma membrane (Miwa *et al.,* 2007). BOR4 was found to be involved in high B tolerance by excluding B out of root cells.

As root cells absorb B, this must be exported from endodermal, pericycle, or xylem parenchyma cells into the stellar apoplasm (xylem loading). Plants developed in media with enough B where xylem loading of B is performed by diffusion across

lipid bilayer and facilitated permeation via protein channel (Dannel *et al.*, 2002). However, energy dependent high affinity transport system mediated via specific BOR transporters is induced in response to low supply of B. Plate I depicts the transport of B in root cells of *Arabidopsis thaliana* under low and high level of B. Under B-limitation, NIP5;1 (for B uptake into roots) and BOR1 (for xylem loading of B) co-ordinately drive efficient B transport across roots into xylem. BOR1 is likely to generate a concentration gradient between root cells and the medium. This concentration gradient is essentially required by NIP5;1 to facilitate B uptake into root cells because NIP5;1 is likely to be a boric acid channel. Miwa and Fujiwara (2010) reported that amounts of B uptake into roots were increased in wild type plants under low level of B compared to those under high level of B condition whereas, this increase of B uptake was not observed in the *nip5;1* mutant. These observations demonstrated that NIP5;1 is essential for B uptake into root cells to support normal plant growth under B limitation. Similar to aquaporins, NIP5;1 is likely to transport boric acid according to concentration gradient, and contributes to satisfy B requirement in shoot and root growth (Takano *et al.*, 2006). Moreover, under high levels of B supply, expression of both NIP5;1 and BOR1 is decreased by transcriptional and post-transcriptional regulation, respectively. BOR1 is incorporated into endosomes and transported to the vacuole for degradation and thus, accumulation of BOR1 protein decreases in response to a high B concentration in the medium. Therefore, downregulation of NIP5;1 and BOR1 might be beneficial for avoidance of overloading of high concentrations of B to shoots (Takano *et al.*, 2005).

The overexpressions of BOR1 improve shoot growth, but not root growth under low B condition. This is reasonable as BOR1 is a transporter for xylem loading (Takano *et al.*, 2002). The plants with enhanced expression of NIP5;1 exhibited improved root elongation under low B conditions (Kato *et al.*, 2009). Overexpression of BOR1 and NIP5;1 resulted in plants with high tolerance to low level of B. These represent the first successful improvement of B deficiency tolerance through modification of transporters. This also reveals potentials of enhancing expression of a gene related to mineral nutrient channel which help to improve growth under nutrient limiting conditions. Besides this, Miwa *et al.* (2007) generated transgenic *A. thaliana* lines overexpressing BOR4. BOR4 accumulation was enhanced when B concentration in the media is high, a sharp contrast to the case of BOR1 which diminishes under

high B conditions. This transgenic lines overexpressing BOR4 showed remarkable improvement of root and shoot growth under high B conditions whereas, the wild type plant fail to grow right after germination. B concentrations in roots and shoots were decreased in these transgenic plant lines. It is likely that overexpressed BOR4 pumps excess B out of the cell (Miwa *et al.*, 2007).

In summary, three different molecular mechanisms have been described for the transport of boric acid from soil solution towards root cell and xylem loading of B. Thus, depending on B availability, B transport can be regulated by: (i) passive transport across plasmalemma mediated by simple diffusion. This system operates mainly when adequate or excess B is available in the soil (ii) facilitated transport carried out by NIP channel proteins and, (iii) an energy dependent high-affinity transport that is induced in response to low supply of B and mediated via BOR transporters (Tanaka and Fujiwara, 2007).

2.2.2.2 Boron translocation in plants

After being loaded into xylem, B is transported towards shoot in a process mediated by transpiration stream (Shelp *et al.*, 1995). However, B can also be transported via phloem to both vegetative and reproductive tissues (Shelp *et al.*, 1995; Matoh and Ochiai, 2005), although the mobility of B varies among species (Brown and Shelp, 1997). Transport of B through phloem involves the formation of boron-diol complexes with sugar alcohols as transport molecules (Brown and Hu, 1996; Hu *et al.*, 1997). In fact, B can readily bind to cis-hydroxyl groups of sugar alcohols (mannitol and sorbitol), which allow B to be transported through phloem. This is further strengthened by the isolation and characterization of mannitol-boron-mannitol complex from the phloem sap of *Apium graveolens* (Hu *et al.*, 1997). In addition, it has been observed that transgenic tobacco and rice plants with an enhanced sorbitol levels had higher capacity to transport B through phloem towards young tissues and help to overcome B deficiency (Brown *et al.*, 1999). However, B transport via phloem, especially to young tissues, also occurs in plants (sunflower, wheat, canola, lupin, *Arabidopsis*, broccoli) that are not able to produce these types of carbohydrates (Stangoulis *et al.*, 2001b; Takano *et al.*, 2001; Matoh and Ochiai, 2005). Nevertheless, the molecular mechanism involved in the transport of B through phloem is still debatable. Despite the knowledge about molecular mechanisms that mediate B uptake

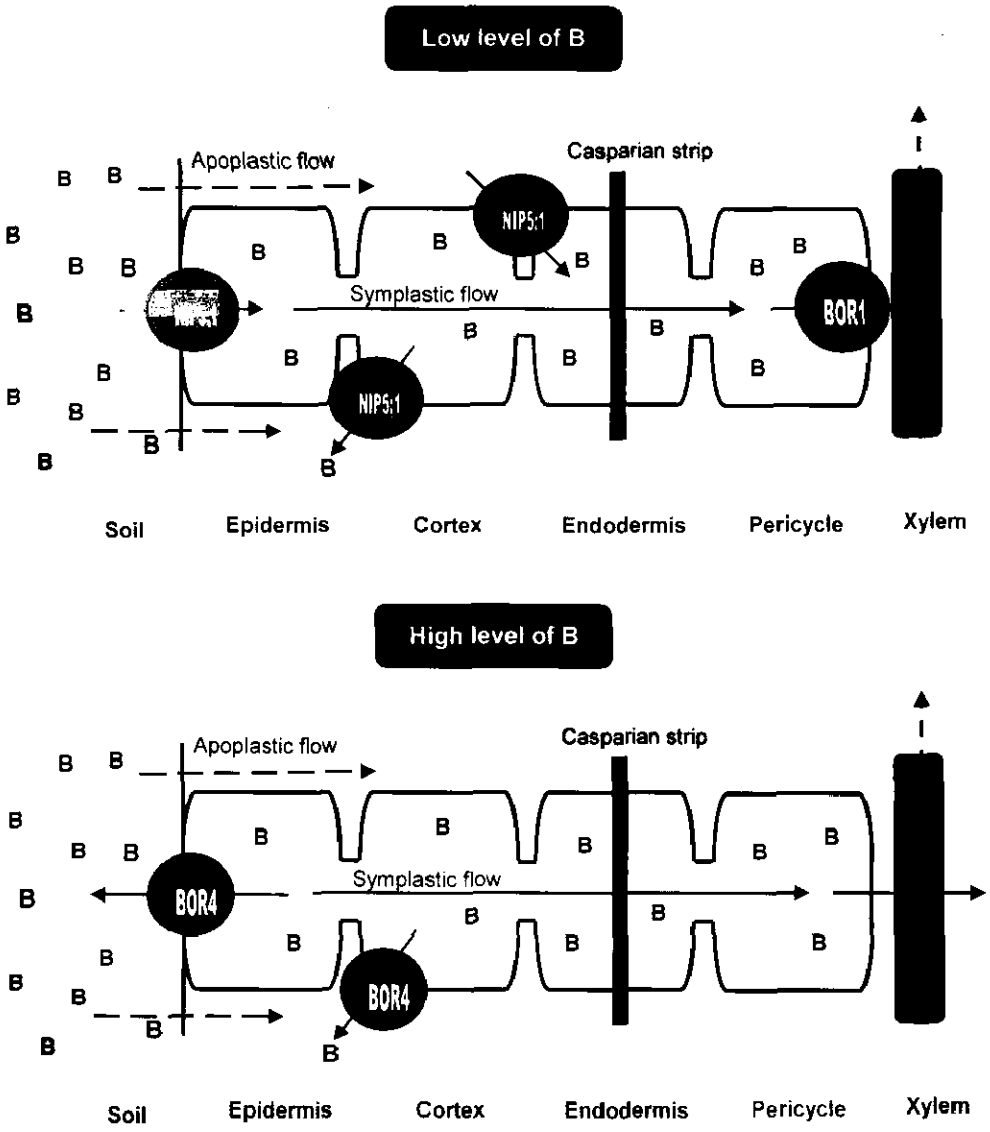

Plate I: A schematic model of boron (B) transport across root cells in *Arabidopsis thaliana*

(Miwa and Fujiwara, 2010)

- ✓ Under low level of B, NIP5;1 and BOR1 co-ordinately drives efficient B transport across root into xylem
- ✓ Under high level of B, NIP5;1 is not strongly expressed and BOR1 degraded. Instead, BOR4 is accumulated to drive efflux of boron from symplasm to soil solution to reduce the concentration of B in roots.

and distribution in plants has advanced notably, however, further investigations are needed to understand better.

2.2.3 Differences in susceptibility of plants to B toxicity

Boron is a unique micronutrient as the window between its deficiency and toxicity is narrow (Yau and Ryan, 2008). Plant tolerance to B toxicity widely varies from species to species (Wu and Dodge, 2005). Moreover, within the species more tolerant varieties could have an endogenous B concentration which is lower than sensitive varieties (Nable *et al.*, 1997; Cervilla *et al.*, 2007; 2012). This behaviour appears to be related to the plant's ability to exclude B at root level: a reduced permeability of membrane lipids and/or the presence of carriers (BOR and NIP) essential for B extrusion from the cytoplasm may be responsible for reduced B accumulation (Miwa *et al.*, 2007; Sutton *et al.*, 2007).

2.2.4 Boron toxicity in plants

Boron toxicity has been recognised as an important worldwide problem limiting crop production in low-rainfall and highly alkaline and saline soils in various regions of the world. In addition, B concentrations build up in the soils as a consequence of over-fertilization and/or irrigation with water containing high levels of B (Nable *et al.*, 1997). B toxicity causes chlorosis starting at the leaf base to margin and tips of mature leaves in many plants which appear as marginal or tip chlorosis or both and necrosis (Nable *et al.*, 1997; Marschner, 1995; Reid *et al.*, 2004; Roessner *et al.*, 2006; Reid and Fitzpatrick, 2009). This presumably results from the accumulation of B, transported through the transpiration stream. Moreover, excess B causes various physiological effects in vascular plants including decreased shoot and root growth (Lovett and Bates, 1984; Nable and Moody, 1990), decreased leaf chlorophyll, inhibition of photosynthesis, lower stomatal conductance (Lovett and Bates, 1984), deposition of lignin and suberin (Ghanati *et al.*, 2002), altered metabolism, increased membrane leakiness, peroxidation of lipids and altered activities of antioxidant pathways (Karabal *et al.*, 2003; Keles *et al.*, 2004). The effects of B toxicity on physiological and metabolic process in vascular plants have been illustrated in plate II. Although the physiological basis for B toxicity is not understood well, however, three main causes have been proposed on the basis of its chemistry (i) alteration of cell wall structure, (ii) metabolic disruption by binding to the ribose moieties of molecules such as ATP, NADH or NADPH, and (iii) disruption of cell division and

development by binding to ribose, either as the free sugar or within RNA (Reid *et al.*, 2004). There is no evidence to support the hypothesis that toxicity in leaves is due to osmotic stress induced by the accumulation of B (Reid *et al.*, 2004). Moreover, growth of the plant is inhibited when B concentration reaches in the range of 1-5 mM but this inhibition is not attributed either due to energy supply or inhibition of protein synthesis, however, toxicity generated in mature tissues could be due to the cumulative retardation of many cellular processes, including photooxidative stress (Reid *et al.*, 2004). An attempt has been made to sum up the toxic effects of B stress on different physiological and biochemical processes in various crops (Table 3).

Table 3: Effect of boron toxicity on growth and development in different crop plants

Crops/ plant species	Boron treatment	Effect	References
Cucurbita pepo	B (20 or 40 mg L^{-1})	Decreased CO_2 fixation, chlorophyll content, shoot growth and root elongation	Lovatt and Bates, 1984
Actinidia species	B (20, 50, 100, 200, or 500 mM)	Decreased P_N, and increased the volume of intercellular spaces and cell damage	Sotiropoulos *et al.*, 2002
Lycopersicon esculentum and Capsicum annuum	B (0, 0.5, 5, or 50 mg kg^{-1} from H_3BO_3)	Decreased fresh and dry mass. Increased membrane permeability, and proline accumulation	Eraslan *et al.*, 2007a
Triticum aestivum	B (0, 5, 10 or 20 mg B kg^{-1} as H_3BO_3)	Reduced the shoot and root dry mass. Increased the uptake and accumulations of B in shoot and root	Turan *et al.*, 2009
Phaseolus vulgaris	B (20 mg kg^{-1})	Reduced root and shoot growth, increased	Gunes *et al.*, 2009

		membrane permeability, H_2O_2 and MDA content and SOD activity, decreased CAT activity whereas, APX activity remained unchanged	
Cucumis sativus	5 mM H_3BO_3	Inhibited root elongation but induced root curvature. Significantly increased H_2O_2 contents in roots, but MDA content was not affected	Wang *et al.*, 2010a
Hordeum vulgare	B (0 or 20 mg kg^{-1})	Increased H_2O_2, lipid peroxidation and lipoxygenase activity	Inal *et al.*, 2009
Triticum aestivum	B (0.5, 1, 2, 3 or 6 mg kg^{-1} soil)	Decreased fresh and dry matter yield, whereas, proline, H_2O_2 and MDA content increased	Metwally *et. al.*, 2012
Raphanus sativus	B (0, 0.5 or 5 mM)	Decreased growth and increased MDA level, H_2O_2 content, and electrolyte leakage	Siddiqui *et al.*, 2012
Brassica juncea	B (0, 0.33, 3.3, 33 or 330 mM)	Decreased seed germination and vigour index of seeds	Archana and Pandey, 2015
Solanum lycopersicum	0.50 or 2 mM H_3BO_3	Decreased biomass, foliar area and chlorophyll content and increased proline content, phenols, flavonoids and H_2O_2	Cervilla *et al.*, 2012
Triticum	B (0, 0.25, 0.5, 1,	Decreased rate of	Ashagre *et al.*,

aestivum	2, 4, 8 or 16 mg L^{-1})	germination, shoot and root lengths, their fresh and dry mass, root number, root/shoot ratio and seedling vigour index	2014
Cicer arietinum	B (0.05 , 1.6 or 6.4 mM)	Increased B concentration and MDA level in the shoots but decreased Fv/ Fm	Ardic *et al.*, 2009b
Citrus grandis	0 , 10 or 500 mM H_3BO_3	Decreased CO_2 assimilation, chlorophyll content and Fv/Fm	Han *et al.*, 2009
Triticum aestivum	B (0, 20, 40 or 60 mg kg^{-1})	Decreased number of spikes $plant^{-1}$, number of kernels $spike^{-1}$, 1000 seed mass, biological yield, harvest index and seed yield	Zare *et al.*, 2013
Lycopersicon esculentum	B (0.0, 100, 200, 300 or 400 ppm)	Increased the activity of glutamate-oxoloacetate and glutamate-pyruvate transaminases enzymes, and soluble proteins in root and shoot	Abo-Hamad and El-Feky, 2014
Phaseolus vulgaris	B (50, 100 or 300 mM)	Reduced chlorophyll and carotenoid contents, net photosynthetic rate and stomatal conductance but increased B content, internal CO_2 concentration, and MDA	Nagesh *et al.*, 2012

		and H_2O_2 content	
Solanum lycopersicum	B (0.05, 0.5 or 2 mM)	Boosted the amount of B, MDA, H_2O_2, and ascorbate and glutathione concentration but decreased growth	Cervilla *et al.*, 2007

2.2.4.1 Boron toxicity and its effect on seed germination and plant growth and development

Seed germination and seedling growth are the most important phases in the life cycle of plant, but are highly responsive to the existing environment. Various plant species respond differentially to different levels of B. Toxic B concentration induces different physiological effects during the life cycle of vascular plants. Banuelos *et al.* (1999) found that excess B adversely affects germination percentage in corn, tomato, carrots and alfalfa. High B concentration also reduced germination percentage of wheat (Yau and Saxena, 1997), maize (Muhammad *et al.*, 2013), safflower (Ashagre *et al.*, 2014) and mustard (Archna and Panday, 2015). The possible reason for this inhibition in seed germination is due to the accumulation of sugars in cotyledons and embryonic axes of growing seedling with increasing B stress (Archna and Panday, 2015).

Plants exposed to high levels of B had reduced growth of shoots and roots (Nable *et al.*, 1990). It has been reported that decrease in root length is one of the earliest and distinct symptoms of B toxicity (Chantachume *et al.*, 1995, Reid *et al.*, 2004). Boron inhibits root growth primarily through limiting cell elongation rather than cell division (Brown *et al.*, 2002). The inhibition in root elongation may be correlated with a decrease in cell number (Choi *et al.*, 2007). The decrease in root elongation under B stress was observed in *Hordeum vulgare* (Choi *et al.*, 2007) and *Cicer arientum* (Karabal *et al.*, 2003; Ardıc *et al.*, 2009a). It has also been reported that shoot and root fresh and their dry mass decreased with an increase in the concentration of B in various species such as tomato and cucumber (Alpaslan and Gunes, 2001), pepper and tomato (Eraslan *et al.*, 2007a), wheat (Turan *et al.*, 2009), barley (Ayvaz *et al.*, 2012), maize (Muhammad *et al.*, 2013) and safflower (Ashagre *et al.*, 2014). Ayfer *et al.* (2006) reported that the concentration of B in wheat shoot was negatively correlated with shoot dry mass under high B supply.

2.2.4.2 Effect of boron on photosynthesis and related attributes

Information on the effects of B on photosynthetic process is still scarce even if it has been established that excess B inhibits photosynthesis (Han *et al.*, 2008; Guidi *et al.*, 2011; Chen *et al.*, 2012). The reduction in CO_2 assimilation, under B stress could be related to a combination of different factors such as oxidative load, decrease in the activities of photosynthetic enzymes and an impaired electron transport rate (Han *et al.*, 2009). However, the mechanisms involved in the alteration of photosynthesis by B stress have not yet been elucidated. It has been reported that the reduction in photosynthetic rate in plant subjected to excess B was accompanied by an increase in intercellular CO_2 concentration whereas, stomatal conductance remains unaffected (Sotiropoulos *et al.*, 2002). In contrast, others observed a reduction in stomatal conductance (Lovatt and Bates, 1984; Papadakis *et al.*, 2004). Pereira *et al.* (2000) hypothesized that one of the possible reasons for the reduction of photosynthesis under excess B was due to structural damage in thylakoid membranes leading to altered rate of electron transport which could have influenced CO_2 photoassimilation. Boron mediated inhibition of photosynthesis have been reported in several species such as summer squash (Lovatt and Bates, 1984), kiwifruit (Sotiropoulos *et al.*, 2002), citrus (Papadakis *et al.*, 2004; Han *et al.*, 2009; Sheng *et al.*, 2010) and pear (Wang *et al.*, 2011).

The maximum quantum yield of photosystem II (Fv/Fm) significantly decreased in many species under B toxicity (Larsson *et al.*, 1998; Papadakis *et al.*, 2004; Ardic *et al.*, 2009b; Guidi *et al.*, 2011). The reduction of Fv/Fm is related to an increase in F_0 (minimal chlorophyll fluorescence) which is closely related to the structural damage to the thylakoid membranes (Havaux and Lannoye, 1985). The decrease in Fv/Fm ratio indicates that leaves are photoinhibited. It is also well known that under these conditions molecular oxygen can act as an alternative electron acceptor for unused electrons (Velez-Ramirez *et al.*, 2011), which leads to the generation of ROS. B induces alterations in the mesophyll cells which in turn reduce electron transport rate and light utilization. On the other hand, the reduced activity of some enzymes involved in CO_2 assimilation (ribulose-1, 5-bisphosphate carboxylase/oxygenase and fructose-1,6-bisphosphate phosphatase) associated with the reduction in NADPH and ATP utilization (Han *et al.*, 2009), inhibits electron transport rate. Consequently, the reduction in electron transport rate determines an oxidative stress that generates ROS

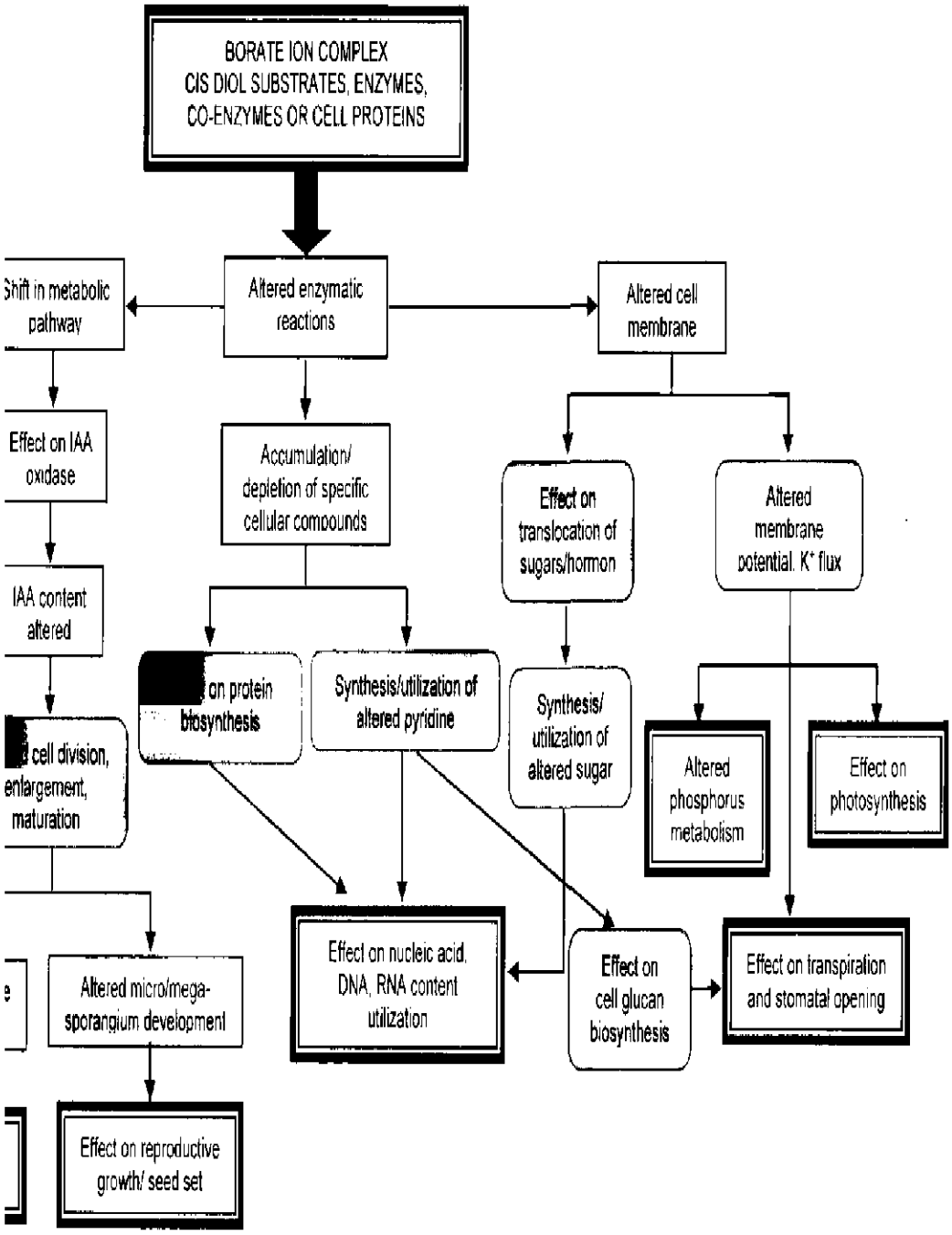

Plate II: Effects of boron toxicity on physiological and metabolic process in higher plants

in the chloroplast. These ROS oxidize organic molecules like chlorophyll and lipid and, probably, induce cell death. These events tend to induce visible symptoms of damage localized at the leaf margin due to the accumulation of B (Guidi *et al.*, 2011).

2.2.4.3 Boron toxicity and antioxidant system

Despite the importance of nutritional disorders, mechanism of B tolerance and toxicity are not properly dissected (Reid *et al.*, 2004; Cervilla *et al.*, 2007). It was suggested that the main tolerance mechanisms could involve: (i) exclusion of B from roots (ii) reduced translocation of B to shoots and, (iii) avoidance by means of shallow root systems (Paull *et al.*, 1992). Genetic variations have been reported in plants to high B concentrations which led to further investigation regarding mechanisms. In wheat and barley plants, several possible tolerance mechanisms of B have been proposed that operate mainly by exclusion (Hayes and Reid, 2004). On the other hand, it has been suggested that antioxidants and antioxidant enzymes could reduce B toxicity in some plants. Modulation of antioxidant system is considered to be a critical process for protecting plants against oxidative damage caused by B toxicity (Gunes *et al.*, 2006; Cervilla *et al.*, 2007).

Boron stress causes an oxidative stress in plants because of increased production of reactive oxygen species (ROS) such as superoxides ($O_2^{\bullet-}$), hydroxy ($OH^{\bullet-}$) and alkoxy ($RO^{\bullet-}$) radicals as induced in many other ionic stresses (Siddiqui *et al.*, 2013; Landi *et al.*, 2014) but the balance between the generation and degradation of ROS is important. Generally, the plants defend against B-induced ROS by the induction of antioxidant enzymes such as catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) and glutathione reductase (GR) etc. which scavenge ROS. Superoxide radicals ($O_2^{\bullet-}$) are toxic by-products of oxidative metabolism and can interact with H_2O_2 to form highly reactive hydroxyl radicals ($OH^{\bullet}$), which are thought to be primarily responsible for oxygen toxicity in the cell (Mittler, 2002; Azevedo-Neto *et al.*, 2006). The oxidative stress triggered by B toxicity increases $O_2^{\bullet-}$ production and, consequently, SOD activity. For this reason, the reaction catalyzed by this enzyme could play an important role in radicals scavenging but, at the same time, other enzymes are necessary to scavenge H_2O_2. Thus, SOD is responsible for detoxification of $O_2^{\bullet-}$ by forming H_2O_2, which is being toxic must be eliminated by conversion to H_2O in subsequent reactions by catalase and several classes of peroxidases. In plants, a number of enzymes regulate H_2O_2 at intracellular levels, but

catalase and ascorbate peroxidase are considered the most important ones (Noctor and Foyer, 1998) and certain specific concentrations of H_2O_2 can promote the activity of catalase and ascorbate peroxidase (Bowler *et al.*, 1992). Once the accumulation of $O_2^{\cdot-}$ and H_2O_2 exceed the scavenging ability of these enzymes, the accumulation of ROS causes lipid peroxidation and membrane damage.

Catalase is responsible for bulk removal of H_2O_2 as they have very high turnover rate but have quite low affinity towards H_2O_2. Catalase activity differs among plant species in response to B toxicity. For instance, B toxicity decreased catalase activity in barley (Inal *et al.*, 2009), citrus (Han *et al.*, 2009) and sunflower (Keles *et al.*, 2011), however, it enhanced the catalase activity in several species such as tomato (Cervilla *et al.*, 2007), chickpea (Ardic *et al.*, 2009 a, b), hot pepper (Lee, 2006) and pear (Wang *et al.*, 2011). The differences in the responses of the same species may be attributed to the different genotypes used in consecutive studies. Peroxidase also plays a crucial role in defence mechanisms against oxidative stress, possibly in co-operation with other antioxidant enzymes, by controlling appropriate H_2O_2 concentrations. Peroxidase activity increased in the leaves of apple root stocks and roots of chick pea respectively, under B treatment (Molassiotis *et al.*, 2006; Ardic *et al.* 2009b).

2.2.5 Combined effect of salinity and excess boron

Stress generated by salinity and B could occur simultaneously when plants are irrigated with water containing high levels of B and salts (Nable *et al.*, 1997), or plants are grown in soils with natural presence of high concentrations of salts and B, usually in semiarid and arid regions characterized by low rainfall and poor drainage (Ben-Gal and Shani, 2002). Table 4 showed the interactive effects of salt and B on growth and development of plants and development

Table 4: Combined effects of salinity and excess B on growth and development in different crops

Crops/ plant species	NaCl and B treatment	Effect	References
Tomato and cucumber	NaCl (0 or 40 mM in tomato; 0 or 30 mM in cucumber) and B	Interaction of B and salinity increased the membrane permeability and decreased growth	Alpaslan and Gunes, 2001

	(0, 5, 10 or 20 mg kg^{-1} soil)	more severely in cucumber than tomato	
Triticum aestivum	2.5 µM H_3BO_3, 75 mM NaCl + 200 µM H_3BO_3 or 75 mM NaCl + 200µM H_3BO_3.	Antagonistic effect was noted for a combined salt and B toxicity regarding concentrations of B and Cl^- whereas, additive effect was observed on shoot fresh mass reduction	Masood *et al.*, 2012
Brassica oleracea	B (1.8 or 4.3 mg L^{-1}) and NaCl (0 or 80 mM)	B and NaCl triggered the responses mediated through aquaporins, together with changes in nutrient transport and plasma membrane stability.	Rodriguez-Hernandez *et al.*, 2013
Capsicum annuum	B (0.046, 0.093, 0.185, 0.370 or 0.740 mM) and NaCl (5, 15, 25, 35 or 45 mM)	Antagonistic relationship has been noted regarding growth and yield of pepper plants under excess B and salinity	Yermiyahu *et al.*, 2008
Brassica oleracia	NaCl (2, 12 or 19 d Sm^{-1}) and B (0.5, 12 or 24 mg L^{-1})	Higher B was relatively more detrimental at low salinity than at higher salinities therefore, tolerance to high levels of B in the presence of salinity was observed. Moreover, reduction in piant head yield and shoot biomass under both salinity and B was noted.	Smith *et al.*, 2010

Capsicum annuum	NaCl (3 or 6 dS m^{-1}) and B (15 or 30 mg kg^{-1})	Combined stress of salinity and B restricted growth than either alone.	Supanjani, 2006
Brassica juncea	NaCl (50 mM) and/or B (1mM)	Growth was more severely affected under the combined treatment than either salinity or high B alone.	Javid *et al.*, 2014
Viburnum tinus	B (1 or 6 mg L^{-1}) and NaCl (2 or 6 dS m^{-1}).	Decreased photosynthesis in presence of combined stress of salt and B	Banon *et al.*, 2012

This simultaneous occurrence of B and salt in soil is being particularly harmful for plants because they suffer from double stress. Salinity increased leaf injury due to B toxicity in tomato and especially in cucumber plants (Alpaslan and Gunes, 2001). Moreover, they observed that membrane permeability of plants was significantly augmented in the presence of salinity by the rising levels of applied B. Also, salinity intensified B toxicity effects since salinity together with boron toxicity increased soluble B concentrations in inter- and intra- cellular compartments of basal leaf sections in wheat when compared to either stress alone, likely related to salinity induced changes in water status (Wimmer *et al.*, 2003). It seems that additive or synergistic relationship could exist between salt stress and B toxicity.

Recently, an antagonistic response has been reported which generates lesser harmful effects of combined salinity and B toxicity than what would be predicted if effects of either stress were additive (Yermiyahu *et al.*, 2008). The possible explanation could be reduced uptake of B in the presence of chloride and reduced uptake of chloride (Cl^-) ions in the presence of B (Yermiyahu *et al.*, 2008). Increased concentration of B in the root could influence the toxicity of NaCl. NaCl toxicity is likely a function of specific Na^+ and/or Cl^- ion toxicity, reduction of K^+ uptake, and osmotic stress. In wheat, concentration of Na^+ in leaves decreased with increasing addition of B to the soil (Holloway and Alston, 1992). They suggested that decreased accumulation of Na^+ was due to the reduction in rooting density caused by B

treatments. Grieve and Poss (2000) also reported that increased B concentration led to low Cl^- ions in leaf of wheat. Yermiyahu *et al.* (2008) also reported that diminution of Cl^- uptake owing to high B could reduce the salt toxicity, since increased addition of B to the soil did not affect leaf Na^+ content in pepper plants. Therefore, B affects salt stress through decreased accumulation of Cl^- in the leaves as a consequence of the reduced Cl^- uptake. It has also been proposed that reduced rates of transpiration would limit accumulation of B in leaf that is transported through the xylem (Yermiyahu *et al.*, 2008). Besides this, increased concentration of NaCl in the root zone could influence the toxicity of B. Letey *et al.* (2001) reported that increased soil salinity might reduce B movement in the broccoli plants and hence results in the loss of B toxicity symptoms. Smith *et al.* (2010) also observed salt-induced reduction of B concentration in shoot under excess B, but its concentration increased under low B supply in broccoli plants. Moreover, reduction of B accumulation in leaves in the presence of salinity may be a function of reduced rates of transpiration in plants where B is transported via xylem.

Another alternative way out is that under simultaneous presence of B and salt stress, the activity of specific membrane components could be influenced by B which could have modulated the functions of certain aquaporin isoforms and ATPase as possible components of salinity tolerance mechanisms (Martinez-Ballesta *et al.*, 2008). In fact, at high external B levels, significant B transport occurs through the plasma membrane aquaporins (Dordas *et al.*, 2000; Dordas and Brown, 2001). Nevertheless, the amelioration mechanism is yet to be dissected in details. Plate III depicts the outline representation of the effects of high concentration of B in the presence of salt stress.

2.3 BRASSINOSTEROIDS

Phytohormones play pivotal role in improving the ability of plants to acclimatize to environmental cues, by modulating growth, development, nutrient distribution and source/sink transitions. Out of well recognised class of phytohormones, brassinosteroids (BRs) is a class of polyhydroxy steroids, placed as sixth group of plant hormone (Bajguz and Hayat, 2009). Till date, about 70 BRs which are structurally and functionally different form each other have been characterized (Bajguz, 2007). Three of them, brassinolide (BL), 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) are being largely applied to have an economic impact on

plant metabolism, growth and productivity with higher stability under field conditions (Khripach *et al.*, 2000; Bajguz and Hayat, 2009). Brassinosteroids are involved in diverse physiological processes such as stem elongation, leaf bending, induction of ethylene, enzymes of photosynthesis and net photosynthetic rate and biosynthesis of nucleic acids and proteins (Khripach *et al.*, 2003; Sasse, 2003; Yu *et al.*, 2004). In addition, BRs also confer tolerance against various abiotic stresses such as those induced by salts, water, drought, low and high temperatures and heavy metals (Fariduddin *et al.*, 2014a; Vardhini and Anjum *et al.*, 2015).

2.3.1 Biosynthesis of brassinosteroids

Brassinosteroids (BRs) biosynthetic pathway is the result of a combination of genetic and biochemical analyses (Fujioka and Yokota, 2003). For the biochemical studies, periwinkle (*Catharanthus roseus*) cell cultures were used, as they produce BRs in relatively higher amounts. Radiolabeled BR intermediates were used in feeding experiments, and their metabolic derivatives were identified by gas chromatography-mass spectroscopy. Coupling this type of analysis to genetic studies of BR-deficient mutants in *Arabidopsis*, tomato, and other species has allowed the identification of complete biosynthetic pathways. BRs are C_{27}, C_{28}, or C_{29} steroids depending on their C-24 alkyl substituents. The most abundant and widely occurring brassinosteroids are C_{28} steroids, and among them brassinolide (BL) is most biologically active and has been found in a large number of plant species (Kim *et al.*, 2008). BL, a 28 carbon molecule possess S-methyl group at C_{24} of the side chain of 5α-ergastane structure, which has been the focus of research on BRs. A simplified version of the BR biosynthetic pathway starts with the sterol progenitor campesterol (CR) (BR-specific). CR is initially converted to campestanol (CN) in several steps. CN is then converted to castasterone (CS) through one of the two pathways called the early and late C-6 oxidation pathways, after which CS is converted to BL (Fujioka *et al.*, 1998). In the early C-6 oxidation pathway, the oxidation at C-6 of the B ring occurs before the hydroxylation at C-22 and C-23 of the side chain (Fujioka *et al.*, 2002). In the late C-6 oxidation pathway, C-6 is oxidised after the introduction of hydroxyls at the side chain and C-2 of the A ring (Taiz and Zeiger, 2006). The early and the late C-6 oxidation pathways coexist and can be linked at different points in *Arabidopsis*, pea, and rice (Fujioka and Yokota, 2003). The available evidences indicate that both the early and late C-6 oxidation pathways are common in plant kingdom, as seen in plants

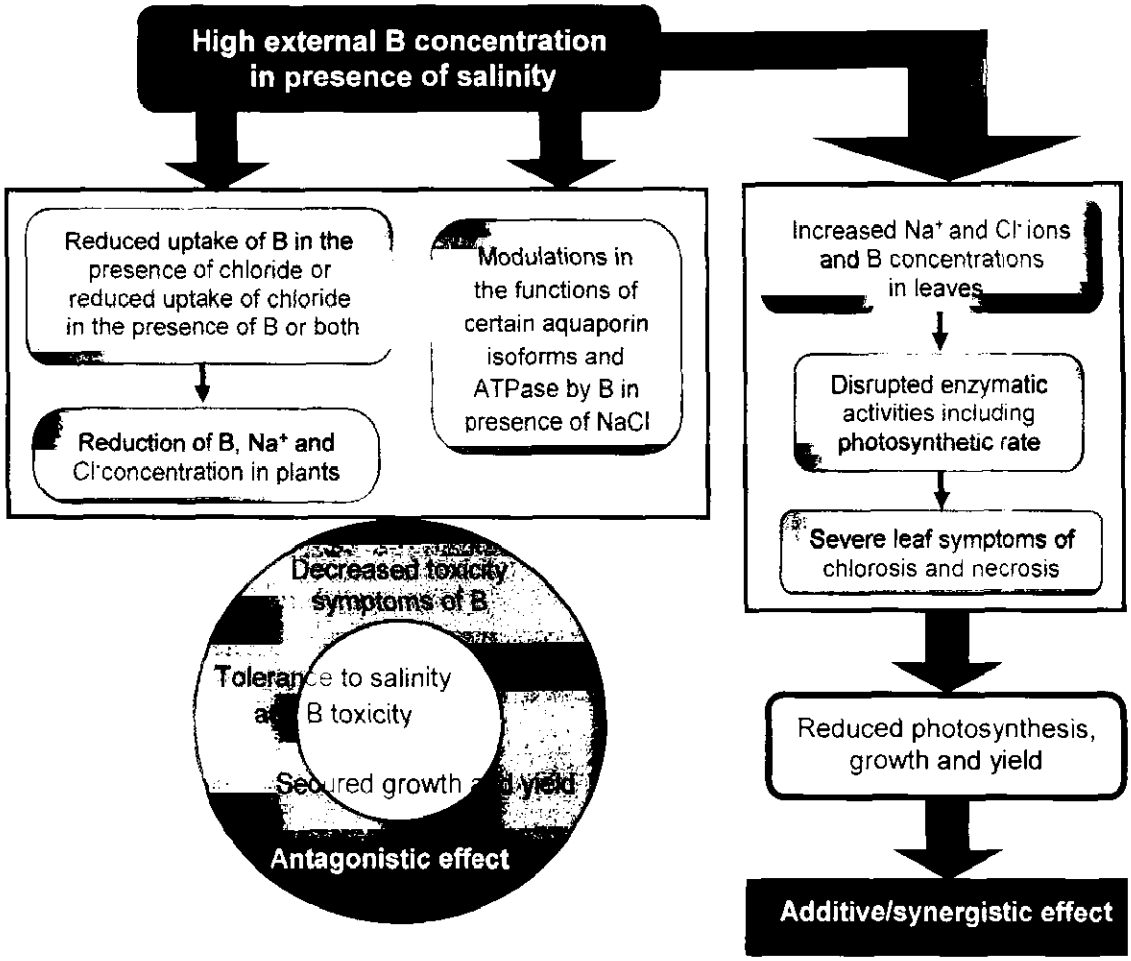

Plate III: Combined effects of salt and B toxicity on plant growth and developme

such as rice (Hong *et al.*, 2002), pea (Nomura *et al.*, 1999). However, in tomato and tobacco, the late C-6 oxidation pathway appears to be the predominant route because the endogenous BRs in these species comprise only members of the late C-6 oxidation pathway (Yokota *et al.*, 2001). In *Arabidopsis*, the late C-6 oxidation pathway is linked at several points to the early C-6 oxidation pathway *via* the BR6ox. Another branching pathway, the early C-22 oxidation pathway was demonstrated using a brassinosteroid-deficient mutant of *A. thaliana*. The presence of several linked pathways increases the complexity of BR biosynthesis and may provide an advantage under different physiological conditions, such as various types of stress. The summary of BR biosynthesis has been illustrated in plate IV.

2.3.2 Signaling of brassinosteroids

Extensive research has been carried out at molecular and biochemical levels to study BR signalling in plants. In the last decade, many key components of the BR signaling pathway have been isolated and characterized. It has been revealed in *A. thaliana* that BR signal transduction pathway starts from ligand perception on the cell membrane to gene expression in the nucleus. BRs are perceived by plasma membrane-localized leucine-rich-repeat receptor kinase, BRI1 (BRASSINOSTERIOD INSENSITIVE1) (Li and Chory, 1997). The auto-phosphorylation and association of BRI1 with second membrane localised receptor BAK1 makes it active after binding to BL. BAK1 is another receptor kinase which acts as a co-receptor with BRI1 (Canodelgado *et al.*, 2004). Due to physical interactions and transphosphorylation of BAK1, it is required to positively regulate BRI1 (Chinchilla *et al.*, 2009). In plants, BR-dependent activation of BRI1 preceded its association with BAK1 which is positively regulated by phosphorylation levels of BAK1 (Wang *et al.*, 2008). BAK1 also transphosphorylates BRI1 increasing its kinase activity towards a specific substrate. However, Li and Jin (2007) reported that BR downstream signalling may be blocked by BKI1 (BRI1 KINASE INHIBITOR1), which inhibits the BRI1 in association with BAK1. Brassinosteroids signaling is summarized in plate V.

In the absence of BRs, BRI1 remains in a state of inactive homodimer owing to its interaction with the cytoplasmic domain, BKI1 (Clouse, 2011; Choudhary *et al.*, 2012). BIN2, a GSK3 kinase repressor protein which is present in nucleus, cytoplasm and plasma membrane phosphorylates two nuclear transcription factors, BZR1 (BRASSINAZOLE RESISTANT1) and BZR2/BES1 (BRI1-EMS SUPRESSOR1),

thereby inhibits their activities. Therefore, BZR1 and BZR2/BES1 association with other proteins or transcription factors is inhibited making them non-functional transcription factors (Vert and Chory, 2006). This BZR1/BES1 phosphorylation catalysed by BIN2 not only prevents DNA binding but also enhances binding to 14-3-3 proteins (phosphopeptide-binding proteins highly conserved in all eukaryotes) (Gampala *et al.*, 2007). However, in the presence of BR, phosphorylation of both BRI1 and BAK1 occurs this induces BR response through the inactivation of BIN2. The inactivation of BIN2 relieves the dephosphorylated forms of BZR1 and BES1. The activated BZR1 and BES1 move into the nucleus to regulate BR-related gene expression directly or via an interaction with other transcription factors (Ryu *et al.*, 2010; Choudhary *et al.*, 2012). Dephosphorylation occurs due to the activity of BSU1 (BRI1 SUPPRESSOR1). The dephosphorylated BES1 and BZR1 activate or inhibit BR-regulated genes. The dephosphorylated BES1 along with three BIM (BES1-interacting Myc-like1) transcription factors bind to E-box motif (CANNTG) to trigger gene expression, whereas BZR1 recognizes the BR response element [CGTG(T/C)G] and acts as a go-between in the feedback inhibition of a number of genes involved in BR biosynthesis (Li and Jin, 2007; Divi and Krishna, 2009).

2.3.3 Physiological roles of brassinosteroids

BRs influence a wide range of important developmental and physiological processes, including regulation of gene expression, cell division and expansion, differentiation, programmed cell death, and homeostasis. The regulation of these processes leads to the promotion of stem elongation and pollen tube growth, leaf bending and epinasty, root growth inhibition, proton-pump activation, and xylem differentiation. Brassinosteroids are implicated in the regulation of numerous developmental and physiological processes. Moreover, BRs delayed senescence in BRs deficient mutants, while accelerated senescence in dying tissue that signifies the biological relevance of BRs action (Clouse and Sasse, 1998). BRs also counteract abiotic stresses in several plants (Sharma and Bhardwaj, 2007; Sharma *et al.*, 2008; Bajguz and Hayat, 2009; Fariduddin *et al.*, 2014a) and inhibit pathogen-associated molecular pattern (PAMP)-triggered immune signaling (Albrecht *et al.*, 2012).

2.3.3.1 Effect of brassinosteroids on seed germination

Seed germination is controlled by a number of mechanisms that are necessary for the growth and development of the embryo, resulting in the eventual production of a

new plant (Miransari and Smith, 2014). It is well documented that BRs promote seed germination like other hormones (Leubner-Metzger, 2003). Endogenous BRs have been identified in the seeds of several species such as pea (Yokota and Takahashi, 1986), *Arabidopsis thaliana* (Schmidt *et al.,* 1997) and *Lychnis viscaria* (Friebe *et al.,* 1999). BRs are able to enhance seed germination by controlling the inhibitory effects of ABA on seed germination (Finkelstein *et al.,* 2008; Zhang *et al.,* 2009). In *Arabidopsis,* it was shown that BR signal reverses the ABA-induced dormancy thus stimulating the germination in *Arabidopsis* (Steber and McCourt, 2001). In *A. thaliana,* BRs also promote the germination of pre-chilled (i.e. non-dormant) seeds of BR-deficient biosynthesis mutant *det2-1* and the BR-insensitive response mutant *bril-1* exposed to light (Steber and McCourt, 2001; Zhang *et al.,* 2009). Seed germination of *det2-1* and *bril-1* are more strongly inhibited by ABA than their wild type. However, application of BRs in rice and *Arabidopsis*, partially revoked the germination inhibited by ABA (Finkelstein *et al.,* 2008; Zhang *et al.,* 2009). Application of 24-epibrassinolide (EBL) resulted in improved seed germination and seedling growth of *Eucalyptus camaldulensis* under saline stress (Sasse *et al.,* 1995). Pre-treatment with BL stimulated the germination and seedling emergence in aged rice grains (Yamaguchi *et al.,* 1987) and seed treatment of barley accelerated subsequent seedling growth (Gregory, 1981). It is, however, not clear whether the promoting effect of BRs in cereal grains is actually manifested only at the level of seedling growth and/or also at the level of germination per se. It has been reported that BRs also promote seed germination in groundnut (Vardhini and Rao, 1997), tobacco (Leubner-Metzger, 2001), tomato (Vardhini and Rao, 2000) barley (Kartal *et al.,* 2009), *Pisum sativum* (Nomura *et al.,* 2007), and *Brassica juncea* (Sirhindi *et al.,* 2009).

2.3.3.2 Effects of brassinosteroids on growth and development

Brassinosteroids play a crucial role in regulating broad spectrum of plant morphological responses such as increased fresh and dry mass of root and shoot, rate of stem elongation, vascular differentiation and photomorphogenesis, proton pump activation, growth of pollen tube, epinastic bending and unrolling of grass leaves at sheath (Clouse and Sasse, 1998). The work with BR biosynthetic mutants in *Pisum sativum* (Nomura *et al.,* 1997) and *Arabidopsis thaliana* (Tao *et al.,* 2004) have provided strong evidences that BRs are essential for plant growth and development

and BR-signaling plays a positive in plant growth and development (Fabregas and Cano-Delgado, 2014). Dwarf and de-etiolated phenotypes and BR-deficient species of some mutants were rescued by application of BRs (Bishop and Yakota, 2001). Friedrichsen *et al.* (2002) also reported that three redundant BR genes encode transcription factors which are required for normal growth, indicating the necessity of BRs for normal growth. Similarly, the inhibition of growth (Asami *et al.,* 2000) and secondary xylem development (Nagata *et al.,* 2001) of *Lepidius sativus* by brassinozole, a specific inhibitor of BL synthesis was reversed by the exogenous application of BL, further emphasizing the necessity of BRs for normal plant growth. Moreover, Mussig *et al.* (2003) reported that exogenous application of BRs along with auxins to BRs deficient mutants of *Arabidopsis* promoted elongation of the root. Kim *et al.* (2007) presented additional evidence that gravitropic bending of root in *Arabidopsis* was mediated by BRs. They also revealed that BRs interacted with auxin differently in root elongation as in gravitropic responses.

2.3.3.3 Brassinosteroids and chlorophyll content

The survey of literature revealed the inductive effect of BRs on the chlorophyll contents in various plant species. However, the level of chlorophyll to some extent depends on the mode of application of BRs. The relationship between exogenously applied BRs and pigment contents in various crop species is summarized in table 5.

Table 5: Effect of brassinosteroids on chlorophyll content

BR analogues with concentration	Mode of application	Crops/ plant species	Response	References
HBL (10^{-8} M)	Foliar spray	*Cicer arientum*	Alleviated the loss of chlorophyll content in Cd stressed seedling	Hasan *et al.*, 2008
HBL and EBL (0.5, 1.0 or 3.0 µM)	Foliar spray	*Raphanus sativus*	Increased level of chlorophyll pigments	Vardhini *et al.*, 2015
BRs (0.5, 1.0 or 2.0 mL L^{-1})	*In vitro* culture of	*Arachis hypogaea*	BR enhanced the chlorophyll	Verma *et al.*, 2012

	seeds		content	
HBL (10^{-10}, 10^{-8} or 10^{-6} M)	Foliar spray	*Satureja khuzestanica*	Elevated the level of chlorophyll	Eskandari and Eskandari, 2013
EBL (5 or 10 µM)	Seed soaking	*Pisum sativum*	Significantly enhanced total chlorophyll content at seedling stage	Shahid *et al.*, 2011
HBL (0.01µM)	Foliar spray	*Vigna radiata*	Increased chlorophyll content at high temperature and/or salt stressed and non-stressed plants	Hayat *et al.*, 2010a
HBL (10^{-10}, 10^{-8} or 10^{-6} M)	Seed soaking	*Brassica juncea*	Increased chlorophyll content under Cu stress	Fariduddin *et al.*, 2009a
HBL (10^{-8}, 10^{-6} or 10^{-5} M)	Foliar spray	*Glycine max*	Increased chlorophyll content	Cevahir *et al.*, 2008
EBL (0.5 mg L^{-1})	Foliar spray	Pepper plant	Ameliorated the adverse effects of salinity and improved chlorophyll content	Houimli *et al.*, 2010
EBL	Foliar spray	*Cucumis sativus*	Significantly increased chlorophyll	Fariduddin *et al.*, 2013

			content	
EBL (10^{-6} or 2×10^{-6} M)	Seed soaking	*Raphanus sativus*	The ratio of chlorophyll (a/b) increased under cadmium stress	Anuradha and Rao, 2009

2.3.3.4 Brassinosteroids and photosynthesis

It is well documented that exogenous application of BRs improved the net photosynthetic rate and its related attributes (Hayat *et al.*, 2010b). The main source of carbon assimilation in plants is photosynthesis. Increasing either photosynthetic efficiency or total plant photosynthetic capacity (delaying leaf senescence) results in the enhanced production of photo-assimilate (Camp, 2005). The most favoured hypothesis is that BRs somehow enhance the efficiency of the photosynthetic carbon reduction cycle by increasing the content of related enzymes. However, it is still not clear what exactly causes BRs-associated changes in photosynthesis and its related attributes. There is a vast of literature available where the effects of BRs on photosynthesis and its related attributes has been examined but a lot of diversity exists among these observations. The information regarding the effect of BRs on photosynthesis and related attributes has been summed up in the table 6.

Table 6: Effect of brassinosteroids on various photosynthetic attributes

BR analogues	Mode of application	Plant species	Response	References
EBL	Foliar spray (0.1 μM)	*Cucumis sativus*	Improved CO_2 assimilation and quantum yield of PSII	Xia *et al.*, 2009
HBL	Foliar spray	*Brassica juncea*	Increased photosynthesis and its related attributes	Fariduddin *et al.*, 2009a, b
EBL/HBL	Seed soaking/ foliar spray	*Oryza sativa*	Enhanced net photosynthetic rate, stomatal conductance and internal CO_2 concentration	Farooq *et al.*, 2009

EBL	Foliar spray (1nM)	*Oryza sativa*	Maintained the maximal quantum efficiency of PSII and net photosynthetic rate	Thussagunpant *et al.*, 2015
BL	Foliar spray (0.1 mg L^{-1})	*Glycine max*	Improved photosynthetic rate, maximum quantum yield of PSII, and Rubisco and carboxylase activity	Zhang *et al.*, 2008
EBL	Foliar spray (0.01 μM)	*Cucumis sativus*	Improved photosynthesis and its related attributes	Fariduddin *et al.*, 2013
EBL	Foliar or root application (50 nM or 5 nM)	*Lycopersicon esculentum*	Significant improvement in photosynthetic attributes under phenanthrene and pyrene phytotoxicity	Ahammed *et al.*, 2012
EBL	Foliar spray	*Lycopersicon esculentum*	Alleviated high temperature-induced inhibition of photosynthesis	Ogweno *et al.*, 2008
HBL	Foliar spray (0.01 μM)	*Brassica juncea*	Protection of photosynthetic machinery under high temperature stress	Fariduddin *et al.*, 2014b
EBL	Foliar spray (0.1 μM)	*Cucumis sativus*	Improved recovery of photosynthetic apparatus from cold stress	Jiang *et al.*, 2012
HBL	Foliar spray (10^{-10}, 10^{-8}	*Satureja khuzestanica*	Increased the rate of photosynthesis	Eskandari and Eskandari,

	or 10^{-6} M)			2013
HBL	Foliar spray	*Triticum aestivum*	Increased maximum quantum yield of PSII (Fv/Fm)	Yusuf *et al.*, 2011
EBL	Foliar spray	*Lycopersicon esculentum*	Improved net photosynthetic rate and Fv/Fm	Wang *et al.*, 2010
EBL	Seed soaking	*Pisum sativum* L.	Significantly increased the photosynthetic rate, stomatal conductance and total chlorophyll contents	Shahid *et al.*, 2011

2.3.3.5 Brassinosteroids and cell differentiation

Recently, it has been shown that BRs play an active role in vascular tissue differentiation. It was also observed that BR increased cell number indicating its role in cell division and differentiation (Clouse and Zurek, 1991). Zurek *et al.* (1994) also showed that BRs play a key role in xylem differentiation in soybean epicotyls. Microscopic studies in BR-deficient mutants have revealed that endogenous BRs play an active role in vascular differentiation (Szekeres *et al.*, 1996). It was revealed that BR deficient *Arabidopsis* mutant *dwf7* had more phloem cells at the cost of xylem cells but number of vascular bundles decreased with irregular spacing (Choe *et al.*, 1999). Exogenous application of BRs overcame the inhibitory effects of uniconazole (a putative BR biosynthesis inhibitor) which inhibits differentiation of mesophyll cells into tracheary elements in *Zinnia elegans* (Iwasaki and Shibaoka, 1991). Yamamoto *et al.* (1997) reported that uniconazole suppressed the transcription of genes related to differentiation which was recovered by BR application.

2.3.3.6 Effects of brassinosteroids on metabolic enzymes

Carbonic anhydrase (CA: 4.2.1.1) is a ubiquitous enzyme which catalyzes the reversible conversion between carbon dioxide and bicarbonate ions (Sultemeyer *et al.*, 1993). CA plays an important role in photosynthetic process (Sultemeyer *et al.*, 1993) and is the second most abundant soluble protein, other than Rubisco in chloroplast of C_3 plants (Okabe *et al.*, 1984). CA has a close association with Rubisco where it

elevates the level of CO_2 at its active site (Badger and Price, 1994). An increase in the activity of CA in the leaves was attained by soaking treatment of EBL to the seeds of *Vigna radiata* (Yusuf *et al.,* 2012) or foliar treatment of HBL to *Brassica juncea* (Hayat *et al.,* 2000, 2001a). Similarly, the application of EBL restored the activity of CA in *Cucumis sativus* by reducing the toxic effect of Cd (Anuradha and Rao, 2009) and salinity (Fariduddin *et al.,* 2013). Moreover, the seedlings of wheat and mung bean raised from the grains treated with HBL possessed high CA activity in their leaves (Hayat *et al.,* 2001b; Fariduddin *et al.,* 2003).

The initial process of reduction of nitrate to ammonia is catalyzed by the enzyme, nitrate reductase (NR: 1.6.6.1). Under various abiotic stress conditions BRs restore the activity of NR enzyme. It plays a pivotal role in the supply of nitrogen and in the growth and productivity of plants. The stress conditions like salinity inhibit the nitrate transport to shoot due to interference with nitrate uptake and xylem loading which is finally attributed to reduced NR activity in leaves (Anuradha and Rao, 2003). Moreover, BRs also played a pivotal role in increasing NR activity in plants, exposed to various stresses viz. wheat (Hayat *et al.,* 2001b), *Lens culinaris* (Hayat and Ahmad, 2003), pea (Shahid *et al.,* 2011) and *Triticum aestivum* (Hayat *et al.,* 2014).

2.3.3.7 Brassinosteroids and antioxidant system

It is evident that BRs are involved in the regulation of reactive oxygen species (ROS) metabolism because they regulate the expression of many antioxidant genes which increase the activity of antioxidant enzymes, like superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) (Cao *et al.,* 2005; Ogweno *et al.,* 2008). BRs have been shown to modulate the stress conditions by regulating the system at the biochemical level. The elevation in the activities of antioxidative enzymes by BRs is a gene regulated phenomenon. It has been demonstrated that *ATPA-2* and *ATP-24* genes, encoding POX were constitutively up-regulated in *DET2Arabidopsis* mutant (Goda *et al.,* 2002). In *Arabidopsis,* the elevation in antioxidative enzyme is a consequence of enhanced expression of *DET2* gene which improved tolerance to oxidative stress (Cao *et al.,* 2005). The enhanced oxidative stress resistance in *DET2* mutant plants was correlated with a constitutive increase in SOD activity and increased transcript levels of the defence gene CAT. Therefore, a possible explanation for the fact that the *DET2* mutant exhibited an enhanced oxidative stress resistance is that the long-term BR deficiency in *DET2* mutant results in a constant *in vivo*

physiological stress that, in turn, activates the constitutive expression of some defence genes and, consequently, the activity of related antioxidant enzymes. It may be suggested that endogenous BRs in wild-type plants somehow represses the transcription or post-transcription activities of the defense genes to ensure normal growth and development of plants. However, it is still unclear whether BRs directly or indirectly modulate the responses of plants to oxidative stress (Cao *et al.,* 2005).

2.3.3.8 Brassinosteroids and plant stress tolerance

Various researchers over the years have indicated the involvement of BRs and associated compounds in plants, exposed to various abiotic stresses such as salinity (Avalbaev *et al.,* 2010; Abbas *et al.,* 2013), drought (Anjum *et al.,* 2011; Li *et al.,* 2012; Mahesh *et al.,* 2013), flooding (Lu *et al.,* 2006; Liang and liang, 2009), light (Wang *et al.,* 2010b; Kurepin *et al.,* 2012), high temperature (Kurepin *et al.,* 2008; Janeczko *et al.,* 2011), low temperature (Divi and Krishna, 2010; Liu *et al.,* 2011; Wang *et al.,* 2014), freezing stress (Janeczko *et al.,* 2009), heavy metal (Arora *et al.,* 2010 a, b; Ashraf *et al.,* 2010; Bajguz, 2010), nutrient stress (Yusuf *et al.,* 2011; Fariduddin *et al.,* 2015), herbicide (Sharma *et al.,* 2013). Therefore, recent reports regarding the role of BRs in the modulation of abiotic stresses in plants are appraised in table 7.

Table 7: Effect of brassinosteroids and abiotic stress tolerance in plants

BR analogues	Abiotic stress	Plant species	Responses	References
EBL	Salinity	*Cucumis sativus*	Improved seedlings growth as a result of increased activities of CAT, POX and SOD	Lu and Yang, 2013
EBL/HBL	Water stress	*Raphanus sativus*	Mediated a reduction in the inhibitory effect of water stress on seed germination and seedling growth by improving the levels of SOD, CAT and APX and free proline	Mahesh *et al.,* 2013

HBL	High temperature and NaCl	*Vigna radiata*	HBL mediated improvement in growth, water relations, photosynthetic machinery and antioxidant system	Hayat *et al.*, 2010a
EBL	Ni	*Raphanus sativus*	Elevated activities of APX, SOD, CAT, GPX, MDHAR, DHAR and GR that eventually resulted in reducing lipid peroxidation, enhanced proline and protein contents, and improved the root/shoot length and fresh biomass	Sharma *et al.*, 2011
EBL	Co	*Brassica juncea*	EBL alleviated the stress generated by Co and significantly increased the activities of SOD, CAT, POX, GR, APOX, MDHAR and DHAR enzymes	Arora *et al.*, 2012
HBL	Zn	*Raphanus sativus*	Conferred tolerance to Zn toxicity by enhancing activities of antioxidative enzymes, strengthening GSH metabolism and redox status, and improving	Ramakrisna and Rao, 2013

			the contents of non-enzymatic antioxidants	
EBL	Cd	*Brassica napus*	EBL reduced the toxic effects of Cd on photochemical processes by diminishing the damage of photochemical reaction centres as well as maintaining efficient photosynthetic electron transport	Janeczko *et al.*, 2005
BR	High temperature	*Oryza sativa*	Exhibited significant enhancement in the expression of POX and SOD, reduction in MDA level and leakage of leaf electrolytes	Cao and Zhao, 2007
EBL	High temperature	*Lycopersicon esculentum*	Significantly alleviated high temperature induced inhibition of photosynthesis by increasing the activities of SOD, APX, GPX, and CAT, and reducing total H_2O_2 and MDA contents	Ogweno *et al.*, 2008

BR	Drought	*Glycine max*	Elevated the activities of POX and SOD, increased the concentration of soluble sugars and proline that eventually resulted into deceased MDA concentration and electrical conductivity	Zhang *et al.*, 2008
EBL	Drought	*Chorispora bungeana*	Conferred tolerance to drought-stress by reducing the lipid peroxidation, membrane permeability as a result of increased activities of antioxidative enzymes and the pools of non-enzymatic antioxidants such as AsA and GSH	Li *et al.*, 2012
HBL	Cd and NaCl	*Triticum aestivum*	Enhanced the photosynthetic related attributes and antioxidant system by reducing the lipid peroxidation and H_2O_2 content	Hayat *et al.*, 2014
EBL	Mn	*Brassica juncea*	Improved growth, water relations and photosynthesis and	Fariduddin *et al.*, 2015

			enhanced various antioxidant enzymes (CAT, POX and SOD)	
BRs (HBL/ EBL)	Cd	*Lycopersicon esculentum*	BRs mediated improvement in photosynthetic machinery and antioxidant system	Hasan *et al.*, 2011
EBL	Zn	*Brassica juncea*	Increased activities of SOD, CAT, APOX, GPX, GR and MDHAR	Arora *et al.*, 2010a
EBL	Cd	*Raphanus sativus*	EBL reduced the toxic effect of Cd on plant growth, pigment content, photosynthesis and enzyme activity	Anuradha and Rao, 2009
EBL	NaCl and Cu	*Cucumis sativus*	Enhanced the activities of various antioxidants	Fariduddin *et al.*, 2013

2.3.3.9 Role of brassinosteroids in amelioration of salt stress

Among the abiotic stress factors, salinity is most important. It is well documented that salinity adversely affects normal plant growth, development, metabolism, cell functioning, etc. Plants have developed many strategies to overcome the ill effects of the salinity. BRs have produced encouraging results in terms of improving plant stress tolerance, including tolerance to salinity stress. The effectiveness of BRs depends on the species, plant developmental stage, concentration used and mode of application (Anzallag, 2002; Fariduddin *et al.*, 2003; Ali *et al.*, 2007). Foliar application of EBL to pepper plants induces tolerance against salinity stress (Samira *et al.*, 2012). Application of EBL as seed soaking increased the seed germination and growth of *Pisum sativum* under saline condition (Shahid *et al.*, 2011). The saline stress also caused a significant reduction in chlorophyll content that may be attributed to the

increased activity of chlorophyll degrading enzyme chlorophyllase, under saline conditions (Reddy and Vora, 1986). HBL application significantly increases the pigment content (Yu *et al.,* 2004; Ali *et al.,* 2007; Hasan *et al.,* 2008). The reason defending the said observation may be the possibility of BRs impact on transcription and/or translation in the synthesis of pigments (Bajguz, 2000). Under salt stress, BRs restore the level of chlorophyll and increase the activity of nitrate reductase enzyme. The activity of this enzyme plays pivotal role in the supply of nitrogen and in turn in the growth and productivity of plants. Salinity inhibits the nitrate transport to shoot due to interference with nitrate uptake and xylem loading which is finally attributed to reduced NR activity in leaves (Anuradha and Rao, 2003). HBL spray to the foliage or supply through roots of *Brassica juncea* plants generated from the seeds soaked in NaCl enhanced the growth, nucleic acid content and seed yield (Hayat *et al.,* 2006, 2007) under normal conditions. BRs have no effect on cell ultrastructure under normal conditions, but significantly reduced the damage to nuclei and chloroplast induced by salt stress (Kulaeva *et al.,* 1991).

BRs are also involved in the protection of the photosynthetic apparatus. BRs, therefore improve photosynthetic rate under different abiotic stresses in mustard (Hayat *et al.,* 2000), mung bean (Fariduddin *et al.,* 2003), wheat (Sairam, 1994). Similarly, the follow up treatment of EBL to salinity stressed pepper plants significantly improved relative growth rate, net CO_2 assimilation, stomatal conductance, transpiration and water use efficiency (Samira *et al.,* 2012). EBL significantly enhances the growth and photosynthetic capacity of salt tolerant and salt-sensitive cultivar of wheat. It has been reported that ameliorative effect of foliar application of BRs was more in salt tolerant wheat cultivars than in salt-sensitive cultivars (Shahbaz *et al.,* 2008). The induced improvement in photosynthetic rate might be due to stomatal and/or non-stomatal factors (Dubey, 2005). In case of non stomatal factors, photosynthetic performance depends largely on the activation state of Rubisco (Yamori *et al., 2006*). The salinity stress inhibits the activity of the key enzymes of photosynthesis viz. Rubisco, PEP-carboxylase and Sedoheptulose-1,7 bisphosphatase (Lefebvre *et al.,* 2005). Under salinity stress, application of BR also protects the quantum yield of PSII in wheat (Shahbaz *et al.,* 2008) and mitigated the inhibitory effect of salt stress on photosynthetic pigments which could be one of the reasons for growth stimulation by BRs under saline conditions (Anuradha and Rao,

2003). Exogenous application of EBL increased the photosynthetic capacity through an increase in quantum yield of PSII (Yu *et al.*, 2004; Yuan *et al.*, 2012).

The pronounced effects of BRs under salinity stress suggested that the elevated level of antioxidant system could have improved the salt tolerance. Such an activity has largely contributed to the protection against oxidative stress, generated by NaCl. Thus, the activity of key antioxidant enzymes under salt-stress can be altered through the exogenous application of BRs (Zhang *et al.*, 2007; Shahbaz *et al.*, 2008). The foliar application of HBL or EBL increased the CAT activity in *Arachis hypogaea* (Vardhini and Rao, 2000) and *Brassica juncea* (Hayat *et al.*, 2000). The effect of foliar application of EBL on the antioxidant system of two wheat cultivars differing in salt tolerance was studied by Shahbaz *et al.* (2008) where it overcome the salinity stress in both the cultivars by increasing POX and CAT activities. Thus, it can be inferred from these results that the applications of BRs has a potential in reducing the toxic effects of salt-stress in different plant species. Furthermore, extensive molecular and physiological research is needed to find out whether the exogenous application of BRs could compensate for the imbalance caused by salt stress and upregulate the defense mechanisms against the stress.

2.3.3.10 Effects of brassinosteroids on senescence

Senescence is a complex and highly regulated process which refers to endogenously regulated deteriorative changes that lead to the natural cause of death of cells, tissues, organs or that of the whole organism (Buchanan-Wollaston, 2007). Maintaining a regulated senescence process is essential for the survival of plants and their future generations. Like other hormones (Rao *et al.*, 2002), BRs also play a crucial role in regulating the processes leading to senescence. The senescence of the leaves of mung bean and mustard supplied with HBL at early stage of growth was delayed (Fariduddin, 2002). However, BRs accelerated senescence in the detached cotyledons of cucumber seedlings (Zhao *et al.*, 1990) in *Xanthium* and *Rumex* explants (Mandava *et al.*, 1981), in leaves of mung bean seedlings (He *et al.*, 1996), and in wheat plant (Saglam-Cag, 2007). However, BR deficient *Arabidopsis* mutants exhibited delayed senescence of chloroplast (Li *et al.*, 1996).

2.3.3.11 Effects of brassinosteroids on crop yield

It is now well evident that BRs increase crop yield. Since the establishment of their presence in plants the possibilities of using these chemicals in improving the yield of

economically useful plants is being explored. Application of BRs was highly effective in enhancing the yield of cucumber (Ikekwa and Zhao, 1991), mustard (Ramraj *et al.*, 1997), *Lens culinaris* (Hayat and Ahmad, 2003), *Vigna radiata* (Fariduddin *et al.*, 2003) and groundnut and tomato (Vardhini and Rao, 2001).

2.4 CONCLUSION AND FUTURE PROSPECTS

Abiotic stresses are the primary cause to loss of productivity, worldwide. Among these stresses, B or salt stress adversely affects normal plant growth, development, metabolism, and finally productivity. B tolerance mechanism of plants has a similarity to salt tolerance mechanism. Similar to NaCl, plant species or genotypes tolerant to B toxicity generally have lower concentrations of B in leaves and shoots than do sensitive species and genotypes. Moreover, the combined effects of B and salt in plant tissues are very complex which could modify ion concentrations and protein composition that affect plasma membrane and cell wall properties. The interplay between B toxicity and salinity is a topic of open debate because varied plant responses to this stress have been reported but do not allow us to achieve a definitive conclusion unless we advance in the understanding of the mechanism by which plants respond simultaneously to both B and salt stresses. Elevation in the generation of ROS is a common consequence in plants growing under NaCl and/or B stress which alter the activity of antioxidant system and adversely affect the process of photosynthesis, growth and yield. Scientists have employed strategies to mitigate the elevated ROS-accrued oxidative stresses/damages via strengthening antioxidant defense system in plants exposed to various abiotic stress. The use of BRs has been considered as a better sustainable alternative for the development of tolerance in plants under adverse conditions of abiotic stress. The response of BRs varies with the plant type and the stage of development. In fact, before any commercial recommendation is made it is important to fix the optimum concentration of BRs and appropriate stage of growth for each of plant species to overcome the stress.

Further, research could unravel the mechanism of action of BRs in stress tolerance and the strategies adopted to increase plant survival in adverse environmental conditions. Since, the discovery of brassinosteroids the mechanism involving steroid perception at cell surface to alterations in gene expression which leads to developmental changes is now much clearly understood (Clouse, 2011). The pleiotropic effects of BRs on physiology of plants appear to be due to BZR1 and

BES1 which regulate the genes to amplify the BR signals. While BES1 and BZR1 appear to be the predominant modulators of BR-regulated gene expression, it is possible that additional, undiscovered transcription factors may be involved in some specific BR responses therefore, searches in future for these proteins may be productive. Furthermore, an improved knowledge of the mechanisms of action of exogenously applied BRs will certainly promote their efficient use in crop production under stressful conditions.

Chapter 3

Materials And Methods

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CHAPTER-3

MATERIALS AND METHODS

3.1 Proposed Study

To achieve the objectives drafted in chapter one, six pot experiments were conducted to explore the effects of two brassinosteroid analogues (28-homobrassinolide and 24-epibrassinolide) in mustard plants [*Brassica juncea* (L.) Czern & Coss] varieties, Varuna and Chapka Rohini, grown under different levels of boron (B) and salinity (NaCl) stress, at selected stages of growth during 2011-2015.

3.2 Seeds

The authentic seeds of *Brassica juncea* (L.) Czern & Coss varieties Varuna and Chapka Rohini were procured from National Seed Corporation Ltd., Pusa, New Delhi, India. Prior to setting up of each experiment, healthy and uniform size seeds were tested for their per cent viability. Viable seeds were surface sterilized with 0.01% mercuric chloride ($HgCl_2$) solution for 5 min, followed by rinsing thrice with double distilled water (DDW), to remove the traces of $HgCl_2$, adhered to the seed surface.

3.3 Preparation of pots

Each earthen pot (25×25 cm) was filled with an equal quantity of sandy loam soil mixed with farmyard manure in a ratio of 6:1. A uniform basal starter dose of inorganic fertilizers (urea, single superphosphate and muriate of potash) was added at a rate of 40 mg, 138 mg and 26 mg respectively, per kg of the soil to each pot to maintain the fertility of the soil. The pots were arranged in a simple randomized block design, in the net house, Department of Botany, Aligarh Muslim University, Aligarh, India.

3.4 Boron treatments

Boron (B) stress was given in the form of boric acid (H_3BO_3). Different concentrations (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B were prepared by adding required amount of H_3BO_3 per kg soil. Deionized water was given to the control plants.

3.5 Salinity treatments

Salinity stress was given in the form of NaCl. Control (non-treated) plants had 1.4 dsm^{-1} of electrical conductivity. The required quantity of NaCl was added to the soil to generate the different levels (1.4, 2.8, 4.2 or 5.6 dsm^{-1}) of salt stress. Deionized water was given to the control plants.

3.6 Hormones and their preparation

28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) were purchased from Sigma-Aldrich India Ltd. Chemicals, India. Stock solutions (10^{-4} M) of both HBL and EBL were prepared by dissolving the required quantity of each hormone in 5 cm^3 of ethanol, in a 100 cm^3 volumetric flask and final volume was maintained up to the mark with DDW. The required lower concentration of HBL or EBL (10^{-8} M) was prepared by diluting the stock solution. The surfactant "tween-20" (0.5 cm^3) was added to each flask prior to treatment.

3.7 Experiment 1

This experiment was performed during the winter season of 2011-12, to study the sensitivity of varied concentrations of boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1}) in two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss, under simple randomized block design. The surface sterilized seeds of both varieties were sown in earthen pots (25×25 cm) at the rate of 10 seeds per pot. A uniform basal dose of N, P, and K was added to the soil, at the time of sowing. The six levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B were generated by adding required quantity of H_3BO_3 to the soil prior to seed sowing. Each treatment was represented by five pots (replicates). Irrigation was done with tap water as and when required. Thinning was done at 10 day after sowing (DAS) to maintain three plants per pot. The plants were harvested, 45 and 60 DAS, to evaluate the following parameters:

1. Length of shoot and root per plant
2. Fresh mass of shoot and root per plant
3. Dry mass of shoot and root per plant
4. Leaf area
5. Chlorophyll value (SPAD level)
6. Net photosynthetic rate (P_N)
7. Stomatal conductance (g_s)
8. Internal CO_2 concentration (C_i)
9. Transpiration rate (E)
10. Maximum quantum yield of photosystem II (Fv/Fm)
11. Leaf electrolyte leakage
12. Leaf carbonic anhydrase (CA) activity
13. Leaf nitrate reductase (NR) activity
14. Leaf catalase (CAT) activity

15. Leaf peroxidase (POX) activity
16. Leaf superoxide dismutase (SOD) activity and
17. Leaf proline content

The remaining plants were allowed to grow up to maturity (approximately 120 DAS) and were harvested to study the following yield characteristics:

18. Number of pods per plant
19. Number of seeds per pod
20. 100 seed mass and
21. Seed yield per plant

3.8 Experiment 2

This experiment was set up according to simple randomized block design during the winter season of 2012-13 to study the effect of lower concentration (2.8 dSm^{-1}) of NaCl alone or in combination with two levels (20 or 60 mg kg^{-1}) of B on two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. The levels of B were selected on the basis of the Experiment 1. The salinity level (2.8 dSm^{-1}) and/or B levels (20 or 60 mg kg^{-1}) were generated by adding required quantity of salt and H_3BO_3 to the soil prior to seed sowing. All other agronomic and cultural practices remained the same as described in Experiment 1. Three plants per pot were maintained and each treatment was represented by five pots. The schematic representation of the treatments is given in table 1.

Table 1: Scheme of treatment for Experiment 2

Treatment	NaCl level (dSm^{-1})	Boron level (mg kg^{-1})
T_1 (Control)	-	-
T_2	2.8	-
T_3	-	20
T_4	-	60
T_5	2.8	20
T_6	2.8	60

A selected number of plants were sampled, 45 and 60 DAS to assess the parameters as studied in Experiment 1. The remaining plants were allowed to grow up to maturity and were harvested, at about 120 DAS to study the yield characteristics.

3.9 Experiment 3

This experiment was also conducted during the winter season of 2012-2013 under simple randomized block design to study the effect of moderate concentration (4.2 dSm^{-1}) of NaCl alone or in combination with two levels (20 or 60 mg kg^{-1}; selected on the basis of Experiment 1) of B in two varieties (Varuna and Chapka Rohini) of mustard (*Brassica juncea*). All the agronomic and cultural practices remained the same as described in Experiment 1. Three plants were maintained in each pot and each treatment was replicated five times. The schematic representation of the treatments is presented in table 2.

Table 2: Scheme of treatment for Experiment 3

Treatment	NaCl level (dSm^{-1})	Boron level (mg kg^{-1})
T_1 (Control)	-	-
T_2	4.2	-
T_3	-	20
T_4	-	60
T_5	4.2	20
T_6	4.2	60

3.10 Experiment 4

This experiment was performed according to simple randomized block design during the winter season of 2013-2014 to study the effect of higher concentration (5.6 dSm^{-1}) of NaCl in the presence or absence of B (20 or 60 mg kg^{-1}) on two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. These two levels of B were selected on the basis of Experiment 1. All the agronomic and cultural practices remained the same as described in Experiment 1. Three plants were maintained in each pot and each treatment was replicated five times. The schematic representation of the treatments is presented in table 3.

Table 3: Scheme of treatment for Experiment 4

Treatment	NaCl level (dSm^{-1})	Boron level (mg kg^{-1})
T_1 (Control)	-	-
T_2	5.6	-
T_3	-	20
T_4	-	60
T_5	5.6	20
T_6	5.6	60

Three plants per pot were maintained after thinning. Plants were irrigated with tap water as and when required. A selected number of plants were sampled at 45 and 60 DAS to assess the various parameters and the remaining plants were allowed to grow up to maturity and were harvested, at about 120 DAS to study the yield traits as studied in Experiment 1.

3.11 Experiment 5

This experiment was performed according to simple randomized block design during the winter season of 2013-14 to study the effect of 10^{-8} M of BR analogues (HBL or EBL) against B stress in two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. The concentrations (20 or 60 mg kg^{-1}) of B were selected on the basis of Experiment 1. The foliage of 44 day old plants was then sprayed with DDW or 10^{-8} M of BRs (HBL/EBL), to elucidate the differential role of HBL/EBL on soil applied B induced changes in mustard varieties. All the agronomic and cultural practices were maintained the same as described in Experiment 1. Three plants were maintained in each pot and each treatment was replicated five times. The scheme of the treatments is presented in table 4.

Table 4: Scheme of treatment for Experiment 5

Treatment	Boron level (mg kg^{-1})	Solution applied to the foliage, 44 DAS
T_1(Control)	-	DDW
T_2	-	HBL (10^{-8} M)
T_3	-	EBL (10^{-8} M)
T_4	20	DDW
T_5	60	DDW
T_6	20	HBL (10^{-8} M)
T_7	60	HBL (10^{-8} M)
T_8	20	EBL (10^{-8} M)
T_9	60	EBL (10^{-8} M)

Each plant was sprinkled three times with an interval of 10 min. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 cm^3 (approx.) in each spray. A group of plants was sampled, at 45 and 60 DAS to assess the parameters as studied in Experiment 1 and the remaining plants were allowed to grow up to maturity

and were harvested after 120 DAS, to study the yield characteristics as mentioned in Experiment 1.

3.12 Experiment 6

This experiment was conducted during the winter season of 2014-15 under simple randomized block design to study the ameliorative effects of the most suited BR analogue (10^{-8} M of EBL; based on the Experiment 5) under combined stress of NaCl and B (selected on the basis of Experiment 2, 3 and 4) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. NaCl and B were amended into the soil before sowing and then the foliage of plants were sprayed with DDW or EBL (10^{-8} M) at 44 DAS. All the agronomic and cultural practices were maintained the same as described in Experiment 1. Each treatment had five replicates (pots) and in each pot three plants were maintained. The scheme of the treatments is presented in table 5.

Table 5: Scheme of treatment for Experiment 6

Treatment	NaCl level (dSm^{-1})	Boron level ($mg\ kg^{-1}$)	Solution applied to the foliage, 44 DAS
T_1 (Control)	-	-	DDW
T_2	-	-	EBL (10^{-8} M)
T_3	2.8	60	DDW
T_4	4.2	60	DDW
T_5	5.6	60	DDW
T_6	2.8	60	EBL (10^{-8} M)
T_7	4.2	60	EBL (10^{-8} M)
T_8	5.6	60	EBL (10^{-8} M)

The foliar application of EBL was given as in Experiment 5. At 45 and 60 DAS, a selected number of plants were sampled to assess the parameters as studied in Experiment 1 and the remaining plants were allowed to grow up to maturity and were harvested after 120 DAS to study the yield characteristics.

3.13 Parameters

The details of the methods executed to assess each parameter are described below:

3.13.1 Growth parameters

3.13.1.1 Length of shoot and root per plant

The plants along with the soil were removed from each pot to get the intact roots and dipped in a bucket filled with tap water. The plants were gently stirred and tapped to remove adhering soil particles. This was followed by washing of roots under running tap water. The length of root and shoot was measured by using a meter scale.

3.13.1.2 Fresh and dry mass of shoot and root

The washed plants were gently soaked with blotting sheets to remove the adhering water. The root and shoot of each plant were separated and weighed on an electronic balance to record their respective fresh mass. Plant roots and shoots were subsequently transferred to an oven run at 80°C and left for 48 h after which they were weighed separately to record their dry mass.

3.13.1.3 Leaf area

The upper third fully expanded leaf was selected for the measurement of leaf area. The leaf area was measured by leaf area meter (ADC Bioscientific, Hoddesdon, Herts, UK).

3.13.2 Physiological characteristics

3.13.2.1 Electrolyte leakage

The total inorganic ions leaked out of the leaves were measured by the method described by Sullivan and Ross (1979). Twenty leaf discs were taken in a boiling tube containing 10 cm^3 of deionized water. The contents were heated at 45°C (EC_a) and 55°C (EC_b) for 30 min each in a water bath and respective electrical conductivity was measured by a conductivity meter. The contents in the boiling tube were again boiled at 100°C (EC_c) for 10 min and the electrical conductivity was again recorded. The percentage of electrolyte leakage was calculated by putting the values in the following formula:

Electrolyte leakage (%) = $[(EC_b - EC_a) / EC_a] \times 100$

3.13.2.2 Chlorophyll content (SPAD value)

The chlorophyll content (SPAD value) at each selected stage was measured in fully expanded leaves of the plants by using Minolta Chlorophyll Meter (SPAD-502; Konica Minolta Sensing Inc. Japan).

3.13.2.3 Photosynthesis and related attributes

Net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) at each selected stage, was measured in

fully expanded leaves of the plants by using portable photosynthesis system (LI-COR 6400, LI-COR, Lincoln, NE, USA). The air temperature, relative humidity, CO_2 concentration and PPFD were maintained at 25°C, 85%, 600 µmol mol^{-1} and 800 µmol mol^{-2} s^{-1}, respectively. All the measurements were made between 11:00 am to 1:00 pm under the clear sun light.

3.13.2.4 Maximum quantum yield of photosystem II

Maximum quantum yield of photosystem II (Fv/Fm) was measured by using Leaf Chamber Fluormeter (LI-COR 6400-40, Portable photosynthesis system, LI-COR, Lincoln, NE, USA). All the measurements were carried out at a photosynthetic photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1}, with a constant airflow rate of 500 µmol s^{-1}. The sampled leaves were dark adapted for 30 min prior to each measurement of Fv/Fm.

3.13.3 Biochemical analysis

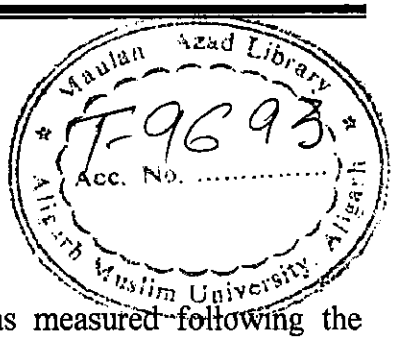
3.13.3.1 Leaf carbonic anhydrase (CA) activity

The carbonic anhydrase (CA) activity (E.C.4.2.1.1) in fresh leaf samples was measured by following the method described by Dwivedi and Randhawa (1974).

The fresh leaf samples were cut into small pieces at a temperature below 25°C. 200 mg of these leaf pieces was weighed and transferred to a petriplates. The leaf pieces were further cuts into fine pieces in 10 cm^3 of 0.2 M cysteine hydrochloride (Appendix 1.1) and were left at 4°C for 20 min and then filtered. The filtrate was transferred to a test tube containing 4 cm^3 of phosphate buffer of pH 6.8 (Appendix 1.2). To this test tube, 4 cm^3 of 0.2 M sodium bicarbonate (Appendix 1.3) solution and 0.2 cm^3 of 0.002% bromothymol blue (Appendix 1.4) were added. The test tube was shaken gently and left at 4°C for 20 min. CO_2 liberated by the catalytic action of carbonic anhydrase on $NaHCO_3$ was estimated by titrating the reaction mixture against 0.5 N HCl (Appendix 1.5) using methyl red as an indicator (Appendix 1.6). The volume of HCl used to develop light purple colour, persisting for at least five seconds was noted. A blank consisting of all the above components of the reaction mixture except the leaf sample was run simultaneously with each set of the samples. The activity of enzyme was calculated by putting the values in the formula:

$$\text{Carbonic anhydrase activity} = \frac{V \times 22 \times N}{W} \quad [\text{mol } (CO_2)\ g^{-1} \text{ (leaf fresh mass)}$$

where, V = Difference in volume (cm^3 of HCl used, in control and test sample titrations)



22 = Equivalent weight of CO_2

N = Normality of HCl

W = Fresh mass of tissue used

3.13.3.2 Leaf nitrate reductase (NR) activity

The activity of nitrate reductase (E.C.1.6.6.1) was measured following the method laid down by Jaworski (1971), in fresh leaf samples.

The leaves were cut into small pieces (1 cm^2). 200 mg of these chopped leaves was weighed and transferred to plastic vials. To each vial 2.5 cm^3 of phosphate buffer (pH 7.4) (Appendix 2.1) and 0.5 cm^3 of potassium nitrate solution (Appendix 2.2) were added, followed by the addition of 2.5 cm^3 of 5% isopropanol (Appendix 2.3). These vials were incubated in a BOD incubator for 2 h at 30 ± 2°C in dark. Incubated mixture (0.4 cm^3) was taken in a test tube to which 0.3 cm^3 each of sulphanilamide solution (Appendix 2.4) and N-1-napthyl-ethylendiamin hydrochloride (NED-HCl) (Appendix 2.5) were added. The test tube was left for 20 min, for maximum colour development. The mixture was diluted to 5 cm^3 by using DDW. The absorbance was read at 540 nm on spectrophotometer (Spectronic-20D, Milton Roy, USA). A blank was run simultaneously with each sample. Standard curve was plotted by using known graded concentrations of $NaNO_2$ (sodium nitrite) solution. The absorbance of each sample was compared with that of the calibration curve and nitrate reductase (NR) activity [n mole NO_2 g^{-1} (FM) s^{-1}] was calculated.

3.13.3.3 Estimation of antioxidant enzyme activity

For the estimation of the activities of antioxidant enzymes, leaf extract was prepared by homogenizing 500 mg of fresh leaves in pre chilled 5 cm^3 of 50 mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrollidone (PVP) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 × g for 10 min at 4°C. The supernatant obtained was further used for biochemical analysis of antioxidant enzymes (catalase, peroxidase and superoxide dismutase).

3.13.3.3.1 Leaf catalase (CAT) activity

The activity of catalase was measured by following the method of Chance and Maehly (1956).

The estimation of catalase was carried out by the permanganate titration method. 3 cm^3 of phosphate buffer (pH 6.8) (Appendix 3.1), 1 cm^3 of 0.1 M H_2O_2 (Appendix 3.2) and 1 cm^3 of enzyme extract were mixed and this mixture was incubated at 25 °C for 1 min. Then 10 cm^3 of 2% H_2SO_4 (Appendix 3.3) was added.

The mixture was titrated against 0.1 N potassium permanganate (Appendix 3.4) to find the residual H_2O_2 until a purple colour persists for at least 15 s. Similarly, a control set was maintained in which the enzyme activity was stopped by the addition of H_2SO_4, prior to the addition of enzyme extract.

3.13.3.3.2 Leaf peroxidase (POX) activity

The activity of peroxidase was also measured by adopting the method of Chance and Maehly (1956).

To 3 cm^3 solution of pyrogallol phosphate buffer (Appendix 4.1), 0.5 cm^3 of 1% H_2O_2 and 0.1 cm^3 of enzyme extract were mixed in a cuvette and a change in absorbance, at 20 s intervals for a period of 3 min was read at 420 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA). The control set was prepared by using all the above reagents, except the enzyme extract.

3.13.3.3.3 Leaf superoxide dismutase (SOD) activity

The activity of superoxide dismutase was measured by the method of Beauchamp and Fridovich (1971).

The reaction mixture (3 cm^3) containing 1 cm^3 of 50 mM phosphate buffer (pH 7.8) (Appendix 5.1), 0.5 cm^3 of 13 mM methionine (Appendix 5.2), 0.5 cm^3 of 75 µM NBT (Appendix 5.3), 0.5 cm^3 of 2 µM riboflavin (Appendix 5.4), 0.5 cm^3 of 0.1 M EDTA (Appendix 5.5) and 0.1 cm^3 of the enzyme extract was prepared. Riboflavin was added at last. The reaction mixture in the tubes were placed under 15 W fluorescent lamps for the initiation of reaction. After 10 min, the reaction was stopped by switching off the light. Non-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA) and the SOD activity was expressed as unit g^{-1} fresh mass. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photo-reduction.

3.13.3.4 Proline content in leaves

The proline content in the fresh leaf samples was measured following the method described by Bates *et al.* (1973).

Fresh leaf sample (0.5 g) was homogenized in a mortar and pestle with 5 cm^3 of 3% sulfosalicylic acid (Appendix 6.1). The homogenate was filtered through Whatman No. 2 filter paper and collected in a test tube with two washings, each with 5 cm^3 of sulfosalicylic acid. 2 cm^3 each of glacial acetic acid and acid ninhydrin (Appendix 6.2) was added to 2 cm^3 of the above extract. This mixture was heated in a

boiling water bath for 1 h. The reaction was terminated by transferring the test tube to ice bath. 4 cm^3 of toluene was added to the reaction mixture with vigorous shaking for 20-30 s. The chromophore (toluene) layer was aspirated and warmed to room temperature. The absorbance of red colour was read at 520 nm against a reagent blank. The amount of proline in the sample was calculated by using a standard curve prepared from pure proline (range 0.1–36 μ mol) and expressed on fresh mass basis of the sample.

$$\mu \text{ moles of proline } g^{-1} \text{ tissues} = \frac{\mu \text{ g proline } cm^{-3} \times cm^{-3} \text{ toluene}}{115.5} \times \frac{5}{\text{g (sample)}}$$

where, 11.5 = the molecular mass of proline

3.13.4 Yield characteristics

3.13.4.1 Number of pods per plant

At harvest (120 DAS), 5 plants from each treatment (representing five replicates) were randomly sampled and counted for the number of pods per plant.

3.13.4.2 Number of seeds per pod

From each treatment, 25 pods were randomly selected and computed to get number of seeds per pod.

3.13.4.3 Seed yield per plant and 100 seed mass

The pods from each replicate were cleaned, crushed, and computed to assess seed yield per plant. 100 seeds were subsequently randomly picked and weighed to record 100 seed mass in mg.

3.14 Statistical analysis

The experiment was conducted according to simple randomized design. Each treatment was represented by five pots where each pot was considered as a replicate. Three plants were maintained per pot. Data was statistically analysed by analysis of variance (ANOVA) using SPSS software version 17 for window (SPSS, Chicago, IL, USA). Least significant difference (LSD) was calculated and 'F' test was applied to assess the significance of the data at $P \leq 0.05$ level of probability.

Chapter 4

Experimental Results

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CHAPTER -4

EXPERIMENTAL RESULTS

4.1 EXPERIMENT 1

This experiment was executed to study the sensitivity to varied concentrations (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of boron (B) supplied through the soil in two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss, under simple randomized block design. The surface sterilized seeds of Varuna and Chapka Rohini of *Brassica juncea* were sown in earthen pots (25×25 cm) at the rate of 10 seeds per pot. A uniform basal dose of N, P, and K was added to the soil at the time of sowing (refer to 3.2). Thinning was done at a particular stage (10 DAS) of growth to maintain three plants per pot. Each treatment was represented by 5 replicates. Irrigation was done with tap water as and when required. The plants were randomly sampled, 45 and 60 DAS, to assess various growth, physiological and biochemical attributes. The rest of the plants were allowed to grow up to maturity and harvested at approximately 120 DAS, to study the yield characteristics.

4.1.1 Shoot and root length

The plants raised from the seeds sown in soil administered with different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) of B had decreased shoot and root length except for 10 mg kg^{-1} in both the varieties (Varuna and Chapka Rohini), at the two stages (45 and 60 DAS) of growth (Table 1). Out of the six tested levels of B, 20 mg B kg^{-1} proved least toxic whereas, 60 mg B kg^{-1} generated maximum toxicity and decreased the shoot and root length by 24% and 31% in Varuna and 39% and 44% in Chapka Rohini, respectively, compared to their respective control plants, at 45 DAS. Early stage (45 DAS) of growth experienced more damage than the later stage (60 DAS). Moreover, the variety Chapka Rohini was more susceptible to the highest level (60 mg kg^{-1}) of B than Varuna, at both the stages of growth.

4.1.2 Shoot and root fresh mass

With the advancement of age from 45 to 60 day stage of growth, the fresh mass of shoot and root increased in both the varieties viz. Varuna and Chapka Rohini (Table 2). However, their mass decreased significantly as the level (20, 30, 40, 50 or 60 mg kg^{-1}) of B in the soil increased, as compared to their respective control plants. The higher concentration (60 mg kg^{-1}) of B proved most deleterious and decreased the values of shoot fresh mass by 26% and 39% and that of root fresh mass by 37% and

Table 2: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	7.90	5.92	6.91	11.95	8.85	10.40	3.12	2.78	2.95	5.36	4.84	5.10
B (10 mg kg^{-1})	8.09	5.94	7.01	11.99	8.87	10.43	3.22	2.91	3.07	5.48	4.98	5.23
B (20 mg kg^{-1})	7.39	5.36	6.38	11.41	8.23	9.82	2.71	2.22	2.46	4.97	4.32	4.65
B (30 mg kg^{-1})	6.97	4.94	5.96	10.99	7.78	9.38	2.56	2.03	2.30	4.46	3.83	4.15
B (40 mg kg^{-1})	6.46	4.14	5.30	10.22	6.82	8.52	2.25	1.68	1.97	4.28	3.59	3.93
B (50 mg kg^{-1})	6.33	4.01	5.17	9.83	6.52	8.18	2.17	1.64	1.90	4.09	3.42	3.76
B (60 mg kg^{-1})	5.85	3.62	4.74	9.11	5.98	7.55	1.96	1.53	1.75	3.62	2.89	3.26
Mean	7.00	4.85		10.79	7.58		2.57	2.11		4.61	3.98	
LSD at 5%	V = 0.21 (Sig.) T = 0.39 (Sig.) V × T = NS			V = 0.40 (Sig.) T = 0.43 (Sig.) V × T = NS			V = 0.02 (Sig.) T = 0.06 (Sig.) V × T = 0.05 (Sig.)			V = 0.15 (Sig.) T = 0.44 (Sig.) V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

45% in Varuna and Chapka Rohini, respectively, in comparison to their unstressed (control) plants, at 45 DAS. However, the intensity of damage was more prominent in Chapka Rohini than Varuna, at both the stages (45 and 60 DAS) of growth. The order of damaging effects of B was 60 mg kg^{-1} > 50 mg kg^{-1} > 40 mg kg^{-1} > 30 mg kg^{-1} > 20 mg kg^{-1} > 10 mg kg^{-1} ≥ Control.

4.1.3 Shoot and root dry mass

Both the varieties (Varuna and Chapka Rohini) showed significant reduction in the shoot and root dry mass in the presence of B (20, 30, 40, 50 or 60 mg kg^{-1}), both at 45 and 60 DAS (Table 3). The decrease in values was more prominent in Chapka Rohini than in Varuna, at both the stages of growth. The concentrations (20 mg kg^{-1} and 60 mg kg^{-1}) of B generated minimum and maximum damage, respectively. Moreover, the highest concentration (60 mg kg^{-1}) of B decreased the shoot and root dry mass in Varuna by 30% and 36% and in Chapka Rohini by 39% and 44% respectively, compared to their respective control plants, at 45 DAS. In addition to this, same concentration (60 mg kg^{-1}) of B decreased the shoot dry mass by 26% and 35% and root dry mass by 31% and 39% in Varuna and Chapka Rohini, respectively, compared to their respective control plants, at 60 DAS, exhibiting a slight recovery in the mass at later growth stage.

4.1.4 Leaf area

The data presented in table 4 revealed that the leaf area increased as growth progressed from 45 to 60 day stage of growth. However, soil applied B (10, 20, 30, 40, 50 or 60 mg kg^{-1}) except 10 mg B kg^{-1} induced a significant reduction of leaf area in both the varieties (Varuna and Chapka Rohini). Out of the tested levels of B, 20 mg kg^{-1} was least toxic whereas, 60 mg kg^{-1} proved highly deleterious. Moreover, the loss generated in leaf area by 60 mg kg^{-1} of B was 28% and 23% in Varuna and 41% and 31% in Chapka Rohini, at 45 and 60 DAS, respectively, compared with their respective control plants. Therefore, Chapka Rohini exhibited greater loss than Varuna at both the stages (45 and 60 DAS) of growth.

4.1.5 Chlorophyll content (SPAD value)

The plants grown in the soil amended with different levels (20, 30, 40, 50 or 60 mg kg^{-1}) of B possessed significantly lower values of SPAD chlorophyll than their control plants in both the varieties i.e. Varuna and Chapka Rohini (Table 4). However, the

Table 3: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on shoot and root dry mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot dry mass						Root dry mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.34	1.74	2.04	3.33	2.50	2.92	0.81	0.62	0.71	1.47	1.03	1.25
B (10 mg kg^{-1})	2.35	1.76	2.06	3.39	2.55	2.97	0.83	0.66	0.74	1.52	1.07	1.29
B (20 mg kg^{-1})	2.11	1.46	1.79	3.10	2.26	2.68	0.71	0.51	0.61	1.34	0.88	1.11
B (30 mg kg^{-1})	1.99	1.31	1.65	2.95	2.06	2.51	0.67	0.45	0.56	1.27	0.78	1.03
B (40 mg kg^{-1})	1.89	1.21	1.55	2.75	1.82	2.29	0.62	0.42	0.52	1.19	0.72	0.95
B (50 mg kg^{-1})	1.82	1.15	1.49	2.69	1.75	2.22	0.60	0.40	0.50	1.14	0.69	0.91
B (60 mg kg^{-1})	1.64	1.06	1.35	2.47	1.63	2.05	0.52	0.34	0.43	1.02	0.64	0.83
Mean	2.02	1.39		2.95	2.08		0.68	0.49		1.28	0.83	
LSD at 5%	V = 0.06 (Sig.)			V = 0.11 (Sig.)			V = 0.02 (Sig.)			V = 0.05 (Sig.)		
	T = 0.12 (Sig.)			T = 0.16 (Sig.)			T = 0.04 (Sig.)			T = 0.07 (Sig.)		
	V × T = 0.17 (Sig.)			V × T = 0.29 (Sig.)			V × T = 0.06 (Sig.)			V × T = 0.13 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 4: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on leaf area (cm^2) and chlorophyll content (SPAD value) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Leaf area						Chlorophyll content (SPAD value)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	36.20	25.46	30.83	44.12	31.23	37.68	43.20	30.24	36.72	57.88	36.97	47.43
B (10 mg kg^{-1})	38.02	26.70	32.36	45.88	32.07	38.98	43.47	30.38	36.93	58.07	37.04	47.56
B (20 mg kg^{-1})	33.67	22.13	27.90	41.98	28.94	35.46	41.47	28.14	34.81	55.67	34.76	45.22
B (30 mg kg^{-1})	31.72	20.57	26.15	39.27	26.11	32.69	40.00	27.36	33.68	53.95	33.67	43.81
B (40 mg kg^{-1})	30.96	19.32	25.14	38.05	24.85	31.45	36.03	23.35	29.69	51.97	30.97	41.47
B (50 mg kg^{-1})	29.69	18.27	23.98	37.48	23.82	30.65	35.06	22.54	28.80	49.63	29.81	39.72
B (60 mg kg^{-1})	26.00	15.03	20.52	34.02	21.41	27.72	32.00	18.76	25.38	44.19	26.21	35.20
Mean	32.32	21.07		40.11	26.92		38.75	25.82		53.05	32.78	
LSD at 5%	V = 1.08 (Sig.)			V = 1.20 (Sig.)			V = 0.20 (Sig.)			V = 0.20 (Sig.)		
	T = 2.01 (Sig.)			T = 2.10 (Sig.)			T = 0.38 (Sig.)			T = 0.82 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.53 (Sig.)			V × T = 0.54 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

maximum loss in the SPAD values of chlorophyll was noted at the highest concentration (60 mg kg^{-1}) of B and the values were reduced by 26% and 38% at 45 DAS and 24% and 29% at 60 DAS, in Varuna and Chapka Rohini, respectively, compared with their corresponding control plants. This decrease was more prominent in Chapka Rohini than Varuna, at both the stages of growth.

4.1.6 Net photosynthetic rate

The values for net photosynthetic rate (P_N) increased as the age of plants advanced from 45 to 60 DAS, in both the varieties (Table 5). Out of the tested concentrations (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B, 10 mg B kg^{-1} did not generate any significant loss. However, the lowest value for net photosynthetic rate was noted in the plants raised with 60 mg kg^{-1} of B and the values were reduced by 31% and 22% in Varuna and 44% and 34% in Chapka Rohini, compared to their respective control plants, at 45 and 60 DAS, respectively.

4.1.7 Stomatal conductance

The increase in the availability of B (20, 30, 40, 50 or 60 mg kg^{-1}) in the soil significantly decreased the values of stomatal conductance (g_s) in both the varieties (Table 5). The highest concentration (60 mg kg^{-1}) of B proved deleterious and decreased the values of g_s by 35% and 45% at 45 DAS and 26% and 40% at 60 DAS, in Varuna and Chapka Rohini, respectively, compared to their control plants. The variety Chapka Rohini was more prone to the B stress than Varuna, at both the growth stages (45 and 60 DAS).

4.1.8 Internal CO_2 concentration

Table 6 shows that the internal CO_2 concentration (C_i) increased as the growth progressed from 45 to 60 DAS. However, the availability of higher levels (20, 30, 40, 50 or 60 mg kg^{-1}) of B in the soil significantly decreased the values of C_i in both the varieties (Varuna and Chapka Rohini). The highest concentration (60 mg kg^{-1}) of B proved toxic which decreased the values of C_i by 25% and 41% in Varuna and 20% and 32% in Chapka Rohini, compared to their control plants, at 45 and 60 DAS, respectively.

4.1.9 Transpiration rate

Transpiration rate (E) significantly decreased in the plants raised in the soil amended with different concentrations (20, 30, 40, 50 or 60 mg kg^{-1}) of B than their control plants, at both the stages (45 and 60 DAS) of growth (Table 6). However, the highest

Table 5: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	19.32	12.40	15.86	26.15	17.56	21.86	0.075	0.046	0.061	0.088	0.058	0.073
B (10 mg kg^{-1})	19.45	12.47	15.96	26.22	17.60	21.91	0.077	0.047	0.062	0.089	0.058	0.074
B (20 mg kg^{-1})	17.52	11.07	14.30	23.98	15.87	19.93	0.068	0.040	0.054	0.083	0.053	0.068
B (30 mg kg^{-1})	16.48	9.96	13.22	22.89	14.86	18.88	0.064	0.035	0.050	0.079	0.051	0.065
B (40 mg kg^{-1})	16.02	9.47	12.75	22.18	14.04	18.11	0.065	0.034	0.050	0.077	0.047	0.062
B (50 mg kg^{-1})	15.00	8.53	11.77	21.35	13.18	17.27	0.061	0.031	0.046	0.074	0.043	0.059
B (60 mg kg^{-1})	13.30	6.92	10.11	20.34	11.63	15.99	0.049	0.025	0.037	0.065	0.035	0.050
Mean	16.73	10.12		23.30	14.96		0.066	0.037		0.079	0.049	
LSD at 5%	V = 0.07 (Sig.)			V = 0.10 (Sig.)			V = 0.001 (Sig.)			V = 0.001 (Sig.)		
	T = 0.14 (Sig.)			T = 0.70 (Sig.)			T = 0.002 (Sig.)			T = 0.002 (Sig.)		
	V × T = 0.19 (Sig.)			V × T = 0.27 (Sig.)			V × T = 0.003 (Sig.)			V × T = 0.003 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 6: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O_2 m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Internal CO_2 concentration (C_i)						Transpiration rate (E)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	344	261	303	375	280	328	3.54	2.53	3.04	4.69	3.46	4.08
B (10 mg kg^{-1})	344	260	302	377	281	329	3.55	2.51	3.03	4.69	3.45	4.07
B (20 mg kg^{-1})	325	240	283	363	263	313	3.39	2.35	2.87	4.53	3.26	3.90
B (30 mg kg^{-1})	321	233	277	353	257	305	3.26	2.27	2.77	4.40	3.15	3.78
B (40 mg kg^{-1})	292	208	250	327	223	275	2.93	1.97	2.45	4.14	2.89	3.52
B (50 mg kg^{-1})	280	193	237	323	217	270	2.90	1.86	2.38	4.13	2.87	3.50
B (60 mg kg^{-1})	257	155	206	301	190	246	2.75	1.63	2.19	3.91	2.60	3.26
Mean	309	221		346	244		3.19	2.16		4.36	3.10	
LSD at 5%	V = 1.16 (Sig.)			V = 1.63 (Sig.)			V = 0.01 (Sig.)			V = 0.02 (Sig.)		
	T = 2.18 (Sig.)			T = 3.05 (Sig.)			T = 0.02 (Sig.)			T = 0.04 (Sig.)		
	V × T = 3.08 (Sig.)			V × T = 4.32 (Sig.)			V × T = 0.03 (Sig.)			V × T = 0.06 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

concentration (60 mg kg^{-1}) of B proved most deleterious and lowered the transpiration rate by 22% and 17% in Varuna and 36% and 25% in Chapka Rohini, compared to their respective control plants, at 45 and 60 DAS, respectively. Moreover, this decrease was more prominent in Chapka Rohini than Varuna, at both the stages of growth.

4.1.10 Maximum quantum yield of PSII

The maximum quantum yield of PSII (Fv/Fm) increased as the growth progressed from 45 to 60 day stage of growth in both the varieties i.e. Varuna and Chapka Rohini (Table 7). Moreover, the variety Varuna possessed higher values for Fv/Fm than Chapka Rohini. Fv/Fm values showed a linear decrease to the increasing concentration (20, 30, 40, 50 or 60 mg kg^{-1}) of B. The maximum loss was noted at the highest concentration (60 mg kg^{-1}) of B which was 26% and 17% less in Varuna and 38% and 27% less in Chapka Rohini, compared to their control plants, at 45 and 60 DAS, respectively.

4.1.11 Electrolyte leakage

Unlike other parameters, leaf electrolyte leakage decreased as growth advanced from 45 to 60 day stage of growth in Varuna and Chapka Rohini (Table 7). However, the presence of B (20, 30, 40, 50 or 60 mg kg^{-1}) in the soil induced a significant increase in the electrolyte leakage as the concentration of B increased. Moreover, the highest concentration of B (60 mg kg^{-1}) increased the electrolyte leakage by 23% and 16% in Varuna and 28% and 22% in Chapka Rohini, at 45 and 60 DAS, respectively, as compared to their control plants. Furthermore, the maximum leakage of electrolytes was noted at 45 day stage of growth, in Chapka Rohini raised with 60 mg B kg^{-1}.

4.1.12 Carbonic anhydrase (CA) activity

The activity of CA increased with the advancement of plant age (45 to 60 day stage of growth). However, the values decreased significantly with the increasing level (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B in the soil, except 10 mg kg^{-1} (Table 8). Out of the six levels of B tested, the highest concentration (60 mg kg^{-1}) was most deleterious which decreased the activity of CA by 21% and 30% at 45 DAS and 18% and 26% at 60 DAS, in Varuna and Chapka Rohini, respectively, compared to their control plants. The variety Varuna possessed higher values for CA activity than Chapka Rohini.

Table 7: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on maximum quantum yield of PSII and electrolyte leakage (%) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Maximum quantum yield of PSII (Fv/Fm)						Electrolyte leakage (%)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	0.845	0.709	0.777	0.979	0.832	0.906	7.49	8.36	7.93	5.94	6.50	6.22
B (10 mg kg^{-1})	0.849	0.712	0.781	0.980	0.832	0.906	7.57	8.48	8.02	5.98	6.56	6.27
B (20 mg kg^{-1})	0.794	0.633	0.713	0.932	0.771	0.852	7.99	9.18	8.58	6.22	6.99	6.61
B (30 mg kg^{-1})	0.785	0.620	0.703	0.922	0.749	0.836	8.24	9.46	8.85	6.39	7.22	6.81
B (40 mg kg^{-1})	0.712	0.544	0.628	0.876	0.683	0.780	8.49	9.82	9.15	6.57	7.45	7.01
B (50 mg kg^{-1})	0.698	0.514	0.606	0.869	0.659	0.764	8.88	10.35	9.62	6.80	7.63	7.22
B (60 mg kg^{-1})	0.628	0.438	0.533	0.816	0.607	0.712	9.24	10.72	9.98	6.91	7.92	7.42
Mean	0.759	0.596		0.911	0.733		8.27	9.48		6.40	7.18	
LSD at 5%	V = 0.004 (Sig.)			V = 0.005 (Sig.)			V = 0.15 (Sig.)			V = 0.10 (Sig.)		
	T = 0.007 (Sig.)			T = 0.010 (Sig.)			T = 0.22 (Sig.)			T = 0.16 (Sig.)		
	V × T = 0.010 (Sig.)			V × T = 0.013 (Sig.)			V × T = 0.29 (Sig.)			V × T = 0.21 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 8: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and nitrate reductase (n mole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Carbonic anhydrase activity						Nitrate reductase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.23	1.76	2.00	2.44	2.00	2.22	438	337	388	570	438	504
B (10 mg kg^{-1})	2.19	1.72	1.96	2.50	2.04	2.27	451	342	397	577	433	505
B (20 mg kg^{-1})	2.13	1.64	1.89	2.37	1.90	2.14	413	309	361	550	416	483
B (30 mg kg^{-1})	2.06	1.53	1.80	2.30	1.81	2.06	380	280	330	536	394	465
B (40 mg kg^{-1})	2.00	1.48	1.74	2.19	1.67	1.93	366	256	311	510	355	433
B (50 mg kg^{-1})	1.96	1.43	1.70	2.11	1.59	1.85	352	247	300	486	339	413
B (60 mg kg^{-1})	1.76	1.24	1.50	2.00	1.48	1.74	316	217	267	442	300	368
Mean	2.05	1.54		2.27	1.78		388	284		524	382	
LSD at 5%	V = 0.04 (Sig.)			V = 004 (Sig.)			V = 10.24 (Sig.)			V = 7.64 (Sig.)		
	T = 0.07 (Sig.)			T = 0.07 (Sig.)			T = 19.15 (Sig.)			T = 22.06 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.10 (Sig.)			V × T = 0.18 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.1.13 Nitrate reductase (NR) activity

The data shown in table 8 revealed that the activity of NR increased in both the varieties (Varuna and Chapka Rohini) as the growth progressed from 45 to 60 day stage. However, increasing the concentration (20, 30, 40, 50 or 60 mg kg^{-1}) of B applied through the soil decreased the activity of NR in a concentration dependent manner. In terms of percentage, the highest concentration (60 mg kg^{-1}) of B decreased the activity of NR by 28% and 22% in Varuna and 36% and 31% in Chapka Rohini, in comparison to their control plants, at 45 and 60 DAS, respectively. Moreover, the decrease in NR activity was more prominent at 45 DAS than at 60 DAS. Out of the two varieties, Chapka Rohini was more vulnerable to B stress than Varuna.

4.1.14 Catalase (CAT) activity

The activity of CAT increased with an increase in the level (20, 30, 40, 50 or 60 mg kg^{-1}) of the B stress and the plant age of both the varieties (Varuna and Chapka Rohini) (Table 9). In terms of percentage, B (60 mg kg^{-1}) increased the CAT activity by 40% and 31% in Varuna and 25% and 18% in Chapka Rohini, compared to their respective control plants, at 45 and 60 DAS, respectively. Moreover, the maximum activity of CAT was noted in Varuna grown in the soil amended with 60 mg kg^{-1} of B, at 45 DAS. Moreover, variety Varuna possessed higher enzyme activity at all the levels of B than Chapka Rohini.

4.1.15 Peroxidase (POX) activity

It is evident from table 9 that the activity of peroxidase, in both the varieties, increased with an increase in the level of B (20, 30, 40, 50 or 60 mg kg^{-1}) in the soil. A maximum increase of 44% and 36% in Varuna and 30% and 18% in Chapka Rohini, compared to their control plants, at 45 and 60 DAS was noted with the highest level of B (60 mg kg^{-1}). Varuna expressed higher activity of POX than Chapka Rohini, at both the stages of growth. Control plants possessed minimum activity of POX, at both 45 and 60 DAS.

4.1.16 Superoxide dismutase (SOD) activity

The activity of SOD also followed a trend similar to that of CAT and POX (Table 10). Plants raised in the soil amended with 60 mg kg^{-1} of B possessed maximum values which was 54% more in Varuna and 39% more in Chapka Rohini than their respective control plants, at 45 DAS. The variety Varuna possessed higher activity of SOD than Chapka Rohini, at both the stages (45 and 60 DAS) of growth.

Table 9: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on catalase (mM H_2O_2 decomposed g^{-1} FM) and peroxidase (units g^{-1} FM) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Catalase activity						Peroxidase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	420	334	377	448	351	400	12.08	8.86	10.47	16.13	11.23	13.68
B (10 mg kg^{-1})	433	337	385	461	356	408	12.51	9.03	10.77	16.36	11.33	13.84
B (20 mg kg^{-1})	459	357	408	480	368	424	13.23	9.57	11.40	17.48	12.02	14.75
B (30 mg kg^{-1})	472	368	420	489	377	433	13.46	9.74	11.60	17.79	12.22	15.00
B (40 mg kg^{-1})	515	386	451	525	389	457	14.29	10.11	12.20	18.84	12.59	15.71
B (50 mg kg^{-1})	526	394	460	554	394	474	14.79	10.48	12.63	19.25	12.81	16.03
B (60 mg kg^{-1})	588	418	503	587	414	501	17.35	11.48	14.42	21.95	13.22	17.59
Mean	488	371		506	378		13.96	9.89		18.26	12.20	
LSD at 5%	V = 4.71 (Sig.)			V = 14.55 (Sig.)			V = 0.29 (Sig.)			V = 0.45 (Sig.)		
	T = 8.82 (Sig.)			T = 20.16 (Sig.)			T = 0.55 (Sig.)			T = 0.78 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.78 (Sig.)			V × T = 1.19 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 10: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on superoxide dismutase activity (units g^{-1} FM) and proline content (µmol g^{-1} FM) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Superoxide dismutase activity						Proline content					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	126	105	116	157	137	147	15.38	12.23	13.81	19.35	16.11	17.73
B (10 mg kg^{-1})	133	108	121	162	139	151	16.12	12.47	14.30	19.74	16.32	18.03
B (20 mg kg^{-1})	141	116	129	172	149	160	17.22	13.48	15.35	21.07	17.12	19.10
B (30 mg kg^{-1})	146	118	132	177	151	164	17.76	13.65	15.71	21.70	17.55	19.62
B (40 mg kg^{-1})	157	125	141	191	157	174	19.81	13.92	16.86	23.90	18.03	20.97
B (50 mg kg^{-1})	165	130	147	197	163	180	21.49	15.63	18.56	24.80	19.07	21.94
B (60 mg kg^{-1})	193	146	170	221	177	199	26.04	18.48	22.26	30.23	22.18	26.21
Mean	152	121		182	153		19.12	14.27		22.97	18.06	
LSD at 5%	V = 4.07 (Sig.)			V = 3.45 (Sig.)			V = 0.53 (Sig.)			V = 0.81 (Sig.)		
	T = 7.40 (Sig.)			T = 8.45 (Sig.)			T = 1.00 (Sig.)			T = 1.22 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.41 (Sig.)			V × T = 2.16 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.1.17 Proline content

It is evident from table 10 that the proline content of the leaves was comparatively higher in the plants that were raised in the soil amended with varied concentration (20, 30, 40, 50 or 60 mg kg^{-1}) of B. The values increased with an increase in the concentration of B as well as the age of the plants. Maximum increase of 69% and 51% was noted with the highest level (60 mg kg^{-1}) of B in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 45 day stage of growth. Out of the two varieties, Chapka Rohini had lower values of proline content than Varuna, at both 45 and 60 DAS.

4.1.18 Yield characteristics

Yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) decreased significantly in response to the soil level (20, 30, 40, 50 or 60 mg kg^{-1}) of B in both Varuna and Chapka Rohini (Tables 11 and 12). The concentration (20 or 60 mg kg^{-1}) of B generated the minimum and maximum values for all the yield characteristics. In terms of percentage, 60 mg B kg^{-1} reduced the number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant by 28%, 19%, 15% and 25% in Varuna and 34%, 27%, 24% and 31% in Chapka Rohini respectively, as compared to their control plants, at harvest (about 120 days after sowing). Varuna possessed higher values than Chapka Rohini.

4.2 EXPERIMENT 2

This experiment was performed with an aim to determine the effect of lower concentration (2.8 dSm^{-1}) of NaCl alone or in combination with two levels (20 or 60 mg kg^{-1}) of B in the same varieties, Varuna and Chapka Rohini (Experiment 1) of *B. juncea* (L.) Czern & Coss. Selection of B concentrations was made on the basis of Experiment 1. All the agronomic and cultural practices were kept the same as described in Experiment 1. The treatments of NaCl and/or B were applied through soil prior to sowing. The plant samples were collected at 45 and 60 DAS to assess various attributes, same as in Experiment 1. The remaining plants were allowed to grow up to maturity and harvested at about 120 DAS, to study the yield characteristics.

4.2.1 Shoot and root length

The shoot and root length improved as the growth progressed from 45 to 60 DAS (Table 13). However, the plants raised in the soil amended with NaCl (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) showed significant reduction in the values, at both the stages (45 and 60 DAS) of growth. Individually, NaCl (2.8 dSm^{-1}) generated toxic

Table 11: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Number of pods per plant			Number of seeds per pod		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	221	183	202	13.25	10.04	11.65
B (10 mg kg^{-1})	228	186	207	13.85	10.12	11.99
B (20 mg kg^{-1})	209	167	188	12.50	9.19	10.85
B (30 mg kg^{-1})	195	150	173	12.18	8.85	10.52
B (40 mg kg^{-1})	186	141	164	11.56	8.11	9.84
B (50 mg kg^{-1})	185	136	161	11.46	7.86	9.66
B (60 mg kg^{-1})	159	120	140	10.69	7.35	9.02
Mean	198	155		12.21	8.79	
LSD at 5%	V = 5.08 (Sig.)			V = 0.52 (Sig.)		
	T = 9.51 (Sig.)			T = 0.27 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 12: Effect of soil applied boron (B; 10, 20, 30, 40, 50 or 60 mg kg^{-1} soil) on 100 seed mass (mg) and seed yield per plant (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	100 seed mass			Seed yield per plant		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	311	270	291	7.28	5.78	6.53
B (10 mg kg^{-1})	324	267	296	7.62	5.96	6.79
B (20 mg kg^{-1})	291	234	263	6.90	5.22	6.06
B (30 mg kg^{-1})	280	223	252	6.46	4.80	5.63
B (40 mg kg^{-1})	274	213	244	6.11	4.36	5.24
B (50 mg kg^{-1})	274	213	244	6.03	4.26	5.15
B (60 mg kg^{-1})	263	205	234	5.48	3.98	4.73
Mean	288	232		6.55	4.91	
LSD at 5%	V = 14.97 (Sig.)			V = 0.22 (Sig.)		
	T = 8.01 (Sig.)			T = 0. 41 (Sig.)		
	V × T = NS			V×T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 13: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root length (cm) in two varieties (Varuna and Chapka rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot length						Root length					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	35.91	27.55	31.73	71.41	59.29	65.35	16.78	11.66	14.22	24.78	18.68	21.73
NaCl (2.8 dSm^{-1})	31.73	21.53	26.63	64.71	48.04	56.37	13.53	8.30	10.92	21.44	14.45	17.95
B (20 mg kg^{-1})	33.73	25.34	29.53	68.48	55.25	61.87	15.12	9.99	12.55	23.38	17.28	20.33
B (60 mg kg^{-1})	27.29	17.06	22.18	57.77	41.34	49.55	11.97	7.11	9.54	19.03	12.33	15.68
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	31.14	21.11	26.13	64.07	47.81	55.94	13.30	8.26	10.78	21.05	14.27	17.66
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	26.93	16.54	21.73	56.49	40.86	48.67	11.88	6.71	9.29	18.77	12.11	15.44
Mean	31.12	21.52		63.82	48.76		13.76	8.67		21.41	14.85	
LSD at 5%	V = 1.04 (Sig.)			V = 1.93 (Sig.)			V = 0.58 (Sig.)			V = 0.62 (Sig.)		
	T = 1.62 (Sig.)			T = 2.65 (Sig.)			T = 1.01 (Sig.)			T = 1.08 (Sig.)		
	V × T = NS			V × T = NS			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

on both shoot and root length which was more than B (20 mg kg^{-1}) alone and decreasing the shoot length by 12% and 22% and root length by 19% and 28% in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 45 DAS. On the other hand, higher concentration (60 mg kg^{-1}) of B alone decreased the shoot length by 24% and 38% and root length by 29% and 40% in Varuna and Chapka Rohini, respectively, in comparison to their control (unstressed) plants, at 45 DAS. Out of the two combinations viz., NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1}) or NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) tested, the maximum reduction was generated by the latter which reduced the shoot length by 25% and 40% at 45 DAS and 21% and 31% at 60 DAS; root length by 29% and 42% at 45 DAS and 24% and 35% at 60 DAS, in Varuna and Chapka Rohini, respectively, compared to their respective control plants. Out of the two varieties, Varuna was more tolerant than Chapa Rohini.

4.2.2 Shoot and root fresh mass

The data depicted in the table 14 indicated that fresh mass of shoot and root increased as the growth progressed from 45 to 60 day stage. However, the plants supplied with NaCl (2.8 dSm^{-1}) alone or with B (20 or 60 mg kg^{-1}) significantly reduced the shoot and root fresh mass as compared to unstressed control plants in both the varieties (Varuna and Chapka Rohini). Individually, the damage caused by NaCl (2.8 dSm^{-1}) in shoot and root fresh mass was less than that of the higher concentration of B (60 mg kg^{-1}). The plants grown in the soil amended with only NaCl (2.8 dSm^{-1}) decreased the shoot and root fresh mass by 16% and 24% in Varuna and 28% and 33% in Chapka Rohini, respectively, compared to their control plants, at 45 DAS. Moreover, among the two combinations, NaCl (2.8 dSm^{-1}) with B (60 mg kg^{-1}) proved most detrimental and decreased the shoot and root fresh mass by 25% and 37% in Varuna and 38% and 47% in Chapka Rohini, respectively, in comparison to their control plants, at 45 DAS. The damage was more pronounced in Chapka Rohini than Varuna.

4.2.3 Shoot and root dry mass

Like the fresh mass, shoot and root dry mass also increased with plant age (Table 15). However, the plants exposed to NaCl (2.8 dSm^{-1}) and/ or B (20 or 60 mg kg^{-1}) significantly reduced the shoot and root dry mass in both the varieties (Varuna and Chapka Rohini). NaCl (2.8 dSm^{-1}) alone decreased the shoot dry mass by 18% and 29% and root dry mass by 26% and 33% in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 45 DAS. Moreover, NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) generated the maximum toxicity and decreased the values of shoot dry mass

Table 14: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	7.69	5.88	6.79	11.59	8.98	10.29	3.16	2.84	3.00	5.30	4.90	5.10
NaCl (2.8 dSm^{-1})	6.43	4.24	5.33	10.17	7.24	8.70	2.41	1.91	2.16	4.30	3.48	3.89
B (20 mg kg^{-1})	7.09	5.27	6.18	10.96	8.23	9.60	2.75	2.27	2.51	4.92	4.37	4.65
B (60 mg kg^{-1})	5.78	3.65	4.72	9.28	6.28	7.78	2.05	1.53	1.79	3.71	2.95	3.33
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	6.32	4.19	5.25	10.04	7.17	8.60	2.37	1.82	2.09	4.23	3.38	3.81
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	5.73	3.64	4.69	9.26	6.19	7.73	1.74	1.19	1.46	3.36	2.62	2.99
Mean	6.51	4.48		10.22	7.35		2.41	1.93		4.30	3.62	
LSD at 5%	V = 0.29 (Sig.)			V = 0.47 (Sig.)			V = 0.11 (Sig.)			V = 0.20 (Sig.)		
	T = 0.51 (Sig.)			T = 0.61 (Sig.)			T = 0.20 (Sig.)			T = 0.35 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.28 (Sig.)			V × T = 0.49 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 15: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root dry mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot dry mass						Root dry mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.29	1.83	2.06	3.36	2.53	2.95	0.82	0.62	0.72	1.51	1.07	1.29
NaCl (2.8 dSm^{-1})	1.88	1.30	1.59	2.90	1.88	2.39	0.60	0.42	0.51	1.24	0.77	1.01
B (20 mg kg^{-1})	2.11	1.56	1.83	3.17	2.32	2.75	0.73	0.51	0.62	1.38	0.91	1.15
B (60 mg kg^{-1})	1.59	1.10	1.34	2.51	1.66	2.09	0.53	0.36	0.45	1.09	0.68	0.88
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	1.81	1.26	1.54	2.85	1.84	2.34	0.58	0.39	0.49	1.19	0.75	0.97
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	1.57	1.10	1.33	2.47	1.63	2.05	0.52	0.35	0.43	1.07	0.67	0.87
Mean	1.87	1.36		2.88	1.98		0.63	0.44		1.25	0.81	
LSD at 5%	V = 0.09 (Sig.)			V = 0.14 (Sig.)			V = 0.02 (Sig.)			V = 0.06 (Sig.)		
	T = 0.16 (Sig.)			T = 0.19 (Sig.)			T = 0.03 (Sig.)			T = 0.10 (Sig.)		
	V × T = 0.23 (Sig.)			V × T = 0.33 (Sig.)			V × T = 0.05 (Sig.)			V × T = 0.14 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

by 31% and 40% at 45 DAS and 26% and 35% at 60 DAS and that of root dry mass by 36% and 44% at 45 DAS and 29% and 37% at 60 DAS, in Varuna and Chapka Rohini, respectively, in comparison to their control plants.

4.2.4 Leaf area

The plants grown in the soil fed with NaCl (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) possessed significantly lower values of leaf area compared to their respective control plants, at both the stages (45 and 60 DAS) of growth (Table 16). Individually, the toxicity triggered by B (60 mg kg^{-1}) was more compared to B (20 mg kg^{-1}) or NaCl (2.8 dSm^{-1}). Moreover, the combination [NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})] induced a maximum loss in leaf area that was 31% and 41% in Varuna and 24% and 34% in Chapka Rohini, lesser than their control plants, at 45 and 60 DAS, respectively. The variety Chapka Rohini was more prone to NaCl and/or B than Varuna.

4.2.5 Chlorophyll content (SPAD value)

Table 16 shows that the chlorophyll content increased as the growth progressed from 45 to 60 day stage. The plants of both the varieties grown in soil administered with NaCl (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) exhibited significantly lower the SPAD value of chlorophyll, compared to their control plants. However, the combination, NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) proved more deleterious than NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1}). Moreover, NaCl with B (20 mg kg^{-1}) or with its higher level (60 mg kg^{-1}) reduced the SPAD value of chlorophyll by 17% and 25% in Varuna and by 29% and 36% in Chapka Rohini, respectively, as compared to their unstressed (control) plants, at 45 DAS. The losses in chlorophyll content were more prominent in Chapka Rohini than Varuna.

4.2.6 Net photosynthetic rate and related attributes

The photosynthetic parameters improved as the growth progressed from 45 to 60 day stage (Tables 17 and 18). However, the plants grown in the soil, supplied with NaCl and/or B had significantly slower rate of net photosynthesis (P_N) and its related parameters [stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E)]. Under NaCl (2.8 dSm^{-1}) stress, the per cent decrease in the values of P_N, g_s, C_i and E was 19%, 21%, 18% and 14% in Varuna and 27%, 30%, 24% and 21% in Chapka Rohini at 45 DAS which was more than the impact of B (20 mg kg^{-1}) but less than that of B (60 mg kg^{-1}). Moreover, NaCl (2.8 dSm^{-1}) in association with B (60 mg kg^{-1}) generated maximum toxicity and reduced the P_N, g_s, C_i, and E values by 32%, 34%, 26% and 21% in Varuna and 45%, 47%, 38% and

Table 16: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on leaf area (cm^2) and chlorophyll content (SPAD value) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Leaf area						Chlorophyll content (SPAD value)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	37.17	25.62	31.40	45.65	34.32	39.99	42.14	29.29	35.72	56.74	35.10	45.92
NaCl (2.8 dSm^{-1})	31.28	18.79	25.03	43.13	31.32	37.23	35.02	21.07	28.05	50.62	28.83	39.72
B (20 mg kg^{-1})	34.40	21.97	28.19	35.10	23.19	29.15	40.03	27.20	33.61	54.23	32.96	43.59
B (60 mg kg^{-1})	26.37	15.25	20.81	40.59	28.11	34.35	31.83	18.96	25.40	44.60	25.09	34.85
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	30.70	17.96	24.33	39.49	26.98	33.24	34.66	20.54	27.60	49.82	28.74	39.28
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	23.78	12.89	18.33	34.62	22.69	28.66	29.99	17.30	23.65	43.75	24.61	34.18
Mean	30.62	18.75		39.76	27.77		35.61	22.39		49.96	29.22	
LSD at 5%	V = 0.90 (Sig.)			V = 1.49 (Sig.)			V = 0.19 (Sig.)			V = 0.18 (Sig.)		
	T = 1.56 (Sig.)			T = 2.58 (Sig.)			T = 0.32 (Sig.)			T = 0.36 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.46 (Sig.)			V × T = 0.45 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 17: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Net photosynthetic rate						Stomatal conductance					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	19.69	12.56	16.13	26.21	17.68	21.95	0.078	0.048	0.063	0.084	0.061	0.073
NaCl (2.8 dSm^{-1})	16.02	9.17	12.59	22.23	13.70	17.97	0.061	0.034	0.047	0.074	0.048	0.061
B (20 mg kg^{-1})	18.12	11.19	14.66	25.13	16.31	20.72	0.071	0.041	0.056	0.080	0.056	0.068
B (60 mg kg^{-1})	13.58	6.94	10.26	20.24	11.76	16.00	0.052	0.027	0.039	0.062	0.037	0.050
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	15.91	8.93	12.42	22.28	13.47	17.87	0.061	0.032	0.046	0.072	0.047	0.059
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	13.29	6.90	10.10	20.14	11.62	15.88	0.047	0.026	0.036	0.061	0.033	0.047
Mean	16.10	9.28		22.70	14.09		0.062	0.035		0.072	0.047	
LSD at 5%	V = 0.11 (Sig.)			V = 0.12 (Sig.)			V = 0.002 (Sig.)			V = 0.002 (Sig.)		
	T = 0.20 (Sig.)			T = 0.26 (Sig.)			T = 0.004 (Sig.)			T = 0.004 (Sig.)		
	V × T = 0.28 (Sig.)			V × T = 0.29 (Sig.)			V × T = 0.005 (Sig.)			V × T = 0.006 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 18: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O_2 m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Internal CO_2 concentration						Transpiration rate					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	342	258	300	377	286	332	3.51	2.63	3.07	4.63	3.48	4.06
NaCl (2.8 dSm^{-1})	281	196	238	333	234	284	3.01	2.08	2.54	4.24	2.93	3.58
B (20 mg kg^{-1})	323	235	279	364	267	316	3.37	2.44	2.91	4.47	3.28	3.87
B (60 mg kg^{-1})	255	162	209	302	203	252	2.75	1.71	2.23	3.93	2.48	3.20
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	275	189	232	325	228	276	2.95	2.04	2.50	4.20	2.90	3.55
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	252	160	206	299	200	249	2.60	1.70	2.15	3.86	2.47	3.16
Mean	288	200		333	236		3.03	2.10		4.22	2.92	
LSD at 5%	V = 1.57 (Sig.)			V = 2.16 (Sig.)			V = 0.01 (Sig.)			V = 0.02 (Sig.)		
	T = 2.71 (Sig.)			T = 3.76 (Sig.)			T = 0.02 (Sig.)			T = 0.03 (Sig.)		
	V × T = 3.84 (Sig.)			V × T = 5.28 (Sig.)			V × T = 0.03 (Sig.)			V × T = 0.05 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

35% in Chapka Rohini, respectively, compared to their respective control plants, at 45 day stage of growth.

4.2.7 Maximum quantum yield of PS II (Fv/Fm)

It is observed from table 19 that the maximum quantum yield of PS II (Fv/Fm) increased as the growth progressed from 45 to 60 day stage. However, plants raised in the soil supplied with NaCl (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) had lower values as compared to unstressed plants of both the varieties. The presence of NaCl (2.8 dSm^{-1}) alone in the soil decreased Fv/Fm by 11% and 17% whereas, the presence of only B (60 mg kg^{-1}) decreased the values by 24% and 35% in Varuna and Chapka Rohini, respectively, as compared to their respective control plants, at 45 DAS. Besides this, the presence of NaCl (2.8 dSm^{-1}) and B (60 mg kg^{-1}) was most toxic that decreased the values by 25% and 16% in Varuna and 35% and 27% in Chapka Rohini, compared to their respective control plants, at 45 and 60 DAS, respectively.

4.2.8 Electrolyte leakage

Electrolyte leakage decreased in both the varieties (Varuna and Chapka Rohini) as the growth progressed from 45 to 60 DAS (Table 19). However, the plants raised in the soil treated with NaCl (2.8 dSm^{-1}) alone or in association with B (20 or 60 mg kg^{-1}) had significantly higher electrolyte leakage, at both the stages (45 and 60 DAS) of growth. The effect of B (60 mg kg^{-1}) and that of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) was comparable and the plants had maximum values of electrolyte leakage. Moreover, the presence of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) increased the electrolyte leakage by 24% and 17% in Varuna and 29% and 22% in Chapka Rohini, compared to their unstressed (control) plants, at 45 and 60 DAS, respectively.

4.2.9 Carbonic anhydrase (CA) and nitrate reductase (NR) activity

The activity of CA and NR was more at later stage (60 DAS) than an early stage (45 DAS) of plant growth (Table 20). However, the plants treated with NaCl (2.8 dSm^{-1}) in the presence or absence of B (20 or 60 mg kg^{-1}) significantly reduced CA and NR activity in both the varieties (Varuna and Chapka Rohini) and the two stages (45 and 60 DAS) of plant growth. The plants grown in the soil supplemented with NaCl (2.8 dSm^{-1}) alone reduced the CA and NR activity by 12% and 16% in Varuna and 20% and 25% in Chapka Rohini, respectively, compared to their control plants, at 45 DAS. However, the combination [NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})] induced a maximum loss in CA and NR activity in the plants, the respective values were 23% and 30% less in Varuna and 27% and 33% less in Chapka Rohini, compared to their control plants, at 45 DAS. The losses in CA and NR activity were more prominent in Chapka Rohini than Varuna.

Table 19: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on maximum quantum yield of PS II and electrolyte leakage (%) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Maximum quantum yield of PS II (Fv/Fm)						Electrolyte leakage (%)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	0.759	0.737	0.748	0.801	0.754	0.778	7.10	8.05	7.58	5.82	6.70	6.26
NaCl (2.8 dSm^{-1})	0.673	0.615	0.644	0.741	0.655	0.698	7.90	9.30	8.60	6.24	7.40	6.82
B (20 mg kg^{-1})	0.721	0.666	0.693	0.763	0.709	0.736	7.57	8.84	8.20	6.10	7.21	6.65
B (60 mg kg^{-1})	0.576	0.481	0.529	0.678	0.554	0.616	8.76	10.32	9.54	6.77	8.16	7.47
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	0.667	0.612	0.639	0.737	0.645	0.691	7.97	9.38	8.68	6.31	7.46	6.88
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	0.569	0.476	0.522	0.670	0.550	0.610	8.81	10.41	9.61	6.82	8.18	7.50
Mean	0.661	0.598		0.732	0.645		8.02	9.38		6.34	7.52	
LSD at 5%	V = 0.004 (Sig.)			V = 0.003 (Sig.)			V = 0.15 (Sig.)			V = 0.15 (Sig.)		
	T = 0.007 (Sig.)			T = 0.008 (Sig.)			T = 0.30 (Sig.)			T = 0.38 (Sig.)		
	V × T = 0.010 (Sig.)			V × T = 0.009 (Sig.)			V × T = 0.47 (Sig.)			V × T = 0.47 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 20: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and nitrate reductase (n mole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Carbonic anhydrase activity						Nitrate reductase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.31	1.80	2.06	2.47	2.08	2.28	440	339	390	580	442	511
NaCl (2.8 dSm^{-1})	2.02	1.44	1.73	2.23	1.66	1.95	367	253	310	520	361	440
B (20 mg kg^{-1})	2.21	1.69	1.95	2.41	1.97	2.19	412	308	360	559	416	488
B (60 mg kg^{-1})	1.82	1.29	1.56	2.00	1.55	1.77	322	230	276	449	307	378
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	2.06	1.42	1.74	2.20	1.64	1.92	362	247	304	506	353	429
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	1.71	1.26	1.49	1.95	1.52	1.73	319	226	273	444	304	374
Mean	2.02	1.48		2.21	1.74		370	267		510	364	
LSD at 5%	V = 0.05 (Sig.)			V = 0.06 (Sig.)			V = 7.58 (Sig.)			V = 16.24 (Sig.)		
	T = 0.07 (Sig.)			T = 0.08 (Sig.)			T = 13.12 (Sig.)			T = 21.80 (Sig.)		
	V × T = 0.13 (Sig.)			V × T = 0.16 (Sig.)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.2.10 Antioxidant enzymes (CAT, POX and SOD) activity

The activity of antioxidant enzymes viz. catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) increased as the growth progressed from 45 to 60 DAS in both the varieties i.e. Varuna and Chapka Rohini (Tables 21 and 22). Moreover, the activity of these enzymes increased further in response to treatment of NaCl (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}). The plants raised in the soil administered with B (60 mg kg^{-1}) alone or NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) generated maximum activity of CAT, POX and SOD, compared to control plants. Moreover, the combination of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) increased the per cent values of CAT by 38% and 29%; POX by 48% and 42%; and SOD by 64% and 49% in Varuna and Chapka Rohini, respectively, compared to their control plants, at 45 day stage of growth. The activity of enzymes was lower in Chapka Rohini than Varuna, at both the stages (45 and 60 DAS) of growth. Control plants possessed minimum activity of the antioxidant enzymes.

4.2.11 Proline content

Like that of antioxidant enzymes, the level of proline also exhibited a significant increase in the presence of NaCl (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}), in both the varieties (Varuna and Chapka Rohini) as compared to their respective control plants (Table 22). In terms of percentage, the treatment of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) increased the proline content by 68% and 54% in Varuna and Chapka Rohini, respectively, compared to their respective unstressed (control) plants, at 45 DAS. The variety Chapka Rohini possessed lower values for the proline content than Varuna, at both the stages (45 and 60 DAS) of plant growth

4.2.12 Yield characteristics

Availability of both NaCl (2.8 dSm^{-1}) and B (20 or 60 mg kg^{-1}) alone or in combination significantly reduced all the yield characteristics (number of pods per plant, number of seeds per pod, mass of 100 seeds and seed yield per plant) more profoundly in Chapka Rohini than Varuna (Tables 23 and 24). Individually, B (60 mg kg^{-1}) was more toxic than NaCl (2.8 dSm^{-1}) or B (20 mg kg^{-1}) which decreased the number of pods per plant, number of seeds per pod, mass of 100 seeds and seed yield per plant by 22%, 19%, 16% and 22% in Varuna and 31%, 27%, 25% and 32% in Chapka Rohini, respectively, compared to their control plants. However, maximum damage was caused by NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) that decreased the number

Table 21: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on catalase (mM H_2O_2 decomposed g^{-1} FM) and peroxidase (units g^{-1} FM) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Catalase activity						Peroxidase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	420	338	379	451	349	400	12.69	8.93	10.81	16.20	11.31	13.76
NaCl (2.8 dSm^{-1})	518	394	456	542	390	466	16.86	10.73	13.80	20.25	12.76	16.50
B (20 mg kg^{-1})	461	361	411	483	366	424	13.88	9.69	11.79	17.50	12.11	14.80
B (60 mg kg^{-1})	572	438	505	574	410	492	18.25	11.68	14.97	22.21	13.57	17.89
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	529	402	465	557	395	476	17.00	11.07	14.04	20.56	12.89	16.72
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	580	442	511	580	423	502	18.76	12.02	15.39	23.00	14.13	18.56
Mean	513	396		531	389		16.24	10.69		19.95	12.79	
LSD at 5%	V = 18.42 (Sig.)			V = 15.28 (Sig.)			V = 0.45 (Sig.)			V = 0.57 (Sig.)		
	T = 30.57 (Sig.)			T = 19.84 (Sig.)			T = 0.78 (Sig.)			T = 0.72 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.10 (Sig.)			V × T = 1.39 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 22: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on superoxide dismutase (units g^{-1} FM) activity and proline content (µmol g^{-1} FM) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Superoxide dismutase activity						Proline content					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	131	112	122	161	145	153	15.46	12.32	13.89	19.48	16.20	17.84
NaCl (2.8 dSm^{-1})	190	143	166	209	171	190	23.37	16.11	19.74	27.31	20.05	23.68
B (20 mg kg^{-1})	147	124	136	177	158	167	17.46	13.44	15.45	21.18	17.49	19.34
B (60 mg kg^{-1})	200	155	178	229	188	209	25.57	18.74	22.15	30.93	22.23	26.58
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	193	146	170	212	173	193	23.96	16.78	20.37	28.32	20.86	24.59
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	215	163	189	240	197	219	26.03	19.03	22.53	30.84	22.80	26.82
Mean	179	141		205	172		21.98	16.07		26.34	19.94	
LSD at 5%	V = 3.98 (Sig.)			V = 6.48 (Sig.)			V = 0.86 (Sig.)			V = 0.80 (Sig.)		
	T = 11.89 (Sig.)			T = 11.22 (Sig.)			T = 1.29 (Sig.)			T = 1.38 (Sig.)		
	V × T = NS			V × T = NS			V × T = 2.11 (Sig.)			V × T = 1.95 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 23: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Number of pods per plant			Number of seeds per pod		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	223	185	204	13.25	10.12	11.69
NaCl (2.8 dSm^{-1})	203	147	175	11.96	8.68	10.32
B (20 mg kg^{-1})	212	170	191	12.57	9.27	10.92
B (60 mg kg^{-1})	174	128	151	10.73	7.35	9.04
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	199	146	172	11.91	8.55	10.23
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	171	124	148	10.53	7.29	8.91
Mean	197	150		11.83	8.54	
LSD at 5%	V = 6.90 (Sig.)			V = 0.69 (Sig.)		
	T = 11.94 (Sig.)			T = 0.74 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 25: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root length (cm) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot length						Root length					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	37.41	28.40	32.91	73.41	60.29	66.85	15.48	10.72	13.10	23.79	19.04	21.42
NaCl (4.2 dSm^{-1})	30.00	19.73	24.86	61.23	44.00	52.62	11.40	7.11	9.25	18.98	13.62	16.30
B.(20 mg kg^{-1})	35.19	26.14	30.66	70.80	56.68	63.74	14.18	9.30	11.74	22.74	17.74	20.24
B (60 mg kg^{-1})	28.83	18.33	23.58	59.54	42.20	50.87	10.83	5.91	8.37	17.64	11.81	14.72
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	29.51	18.95	24.23	59.64	41.38	50.51	11.21	6.89	9.05	18.55	13.13	15.84
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	24.72	16.32	20.52	52.97	37.46	45.22	9.15	5.43	7.29	16.34	10.85	13.60
Mean	30.94	21.31		62.93	47.00		12.04	7.56		19.67	14.37	
LSD at 5%	V = 0.99 (Sig.)			V = 1.71 (Sig.)			V = 0.41 (Sig.)			V = 0.75 (Sig.)		
	T = 1.71 (Sig.)			T = 2.29 (Sig.)			T = 0.70 (Sig.)			T = 1.14 (Sig.)		
	V × T = NS			V × T = NS			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 26: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	7.78	5.89	6.84	11.26	8.54	9.90	3.28	2.79	3.04	5.31	4.85	5.08
NaCl (4.2 dSm^{-1})	6.03	3.83	4.93	9.14	6.15	7.64	2.24	1.73	1.98	3.74	3.12	3.43
B (20 mg kg^{-1})	7.28	5.32	6.30	10.75	7.90	9.32	2.83	2.21	2.52	4.93	4.35	4.64
B (60 mg kg^{-1})	5.85	3.61	4.73	8.94	5.89	7.41	2.04	1.47	1.76	3.60	2.95	3.28
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	5.87	3.72	4.80	9.01	6.00	7.50	2.17	1.67	1.92	3.68	3.05	3.36
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	5.25	3.26	4.25	8.09	5.49	6.79	1.87	1.31	1.59	3.30	2.57	2.94
Mean	6.34	4.27		9.53	6.66		2.41	1.86		4.09	3.48	
LSD at 5%	V = 0.27 (Sig.)			V = 0.37 (Sig.)			V = 0.11 (Sig.)			V = 0.21 (Sig.)		
	T = 0.48 (Sig.)			T = 0.53 (Sig.)			T = 0.14 (Sig.)			T = 0.23 (Sig.)		
	V x T = NS			V x T = NS			V x T = NS			V x T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Moreover, out of the two combinations of NaCl and B, the maximum decline was noted against NaCl (4.2 dSm^{-1}) + B (60 mg kg^{1}) which decreased the shoot fresh mass by 35% and 44%; root fresh mass by 43% and 53% in Varuna and Chapka Rohini, respectively, compared with their control plants, at 45 DAS.

4.3.3 Shoot and root dry mass

The data in table 27 showed that the shoot and root dry mass increased as the growth progressed from 45 to 60 DAS in both the varieties (Varuna and Chapka Rohini). However, the plants raised in the soil supplemented with NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) had lower shoot and root dry mass, as compared to the control plants. NaCl (4.2 dSm^{-1}) decreased the shoot dry mass by 26% and 38% and that of root dry mass by 32% and 40% in Varuna and Chapka Rohini, respectively, as compared to their respective control plants, at 45 DAS. Moreover, combined stress of NaCl (4.2 dSm^{-1}) and B (60 mg kg^{1}) generated maximum toxicity and reduced the shoot dry mass in Varuna and Chapka Rohini by 37% and 47% at 45 DAS and 31% and 39% at 60 DAS and that of root dry mass by 42% and 51% at 45 DAS and 37% and 45% at 60 DAS, respectively, compared to their respective control plants. Two varieties (Varuna and Chapka Rohini) showed contrasting response to stress, at both the stages of growth.

4.3.4 Leaf area

As plant age progressed from 45 to 60 day stage, per cent of the leaf area increased (Table 28). The plants exposed to NaCl (4.2 dSm^{-1}), B (20 mg kg^{-1}) or B (60 mg kg^{-1}) alone or in combination significantly lost leaf area in both the varieties (Chapka and Varuna). NaCl (4.2 dSm^{-1}) alone reduced the leaf area by 27% and 17% in Varuna and 38% and 29% in Chapka Rohini, compared to their control plants, at 45 and 60 DAS, respectively. Moreover, out of the two combinations, NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1}) and NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) tested, the later caused maximum loss in leaf area which was 36% and 27% in Varuna and 49% and 37% lower in Chapka Rohini, at 45 and 60 DAS, respectively, in comparison to their control plants. The damage was more pronounced in variety Chapka Rohini than Varuna at both the growth stages.

4.3.5 Chlorophyll content (SPAD level)

The data shown in table 28 revealed that the SPAD value of chlorophyll increased as the growth progressed from 45 to 60 day stage. However, the availability of NaCl

Table 23: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Number of pods per plant			Number of seeds per pod		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	223	185	204	13.25	10.12	11.69
NaCl (2.8 dSm^{-1})	203	147	175	11.96	8.68	10.32
B (20 mg kg^{-1})	212	170	191	12.57	9.27	10.92
B (60 mg kg^{-1})	174	128	151	10.73	7.35	9.04
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	199	146	172	11.91	8.55	10.23
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	171	124	148	10.53	7.29	8.91
Mean	197	150		11.83	8.54	
LSD at 5%	V = 6.90 (Sig.)			V = 0.69 (Sig.)		
	T = 11.94 (Sig.)			T = 0.74 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 24: Effect of soil applied sodium chloride (NaCl; 2.8 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on 100 seed mass (mg) and seed yield per plant (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	100 seed mass			Yield per plant		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	314	265	290	7.52	5.70	6.61
NaCl (2.8 dSm^{-1})	291	226	259	6.65	4.40	5.53
B (20 mg kg^{-1})	294	231	262	7.15	5.07	6.11
B (60 mg kg^{-1})	263	199	231	5.87	3.85	4.86
NaCl (2.8 dSm^{-1}) + B (20 mg kg^{-1})	287	223	255	6.58	4.34	5.46
NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1})	259	195	227	5.55	3.36	4.45
Mean	285	223		6.55	4.45	
LSD at 5%	V = 11.66 (Sig.)			V = 0.24 (Sig.)		
	T = 16.41 (Sig.)			T = 0.42 (Sig.)		
	V x T = NS			V x T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

of pods per plant, number of seeds per pod, mass of 100 seeds and seed yield per plant by 23%, 20%, 17% and 22% in Varuna and 33%, 28%, 26% and 34% in Chapka Rohini, respectively, compared to their respective unstressed (control) plants, at harvest.

4.3 EXPERIMENT 3

This experiment was set up with an objective to determine the effect of moderate concentration (4.2 dS m^{-1}) of NaCl alone or in association with two concentrations (20 or 60 mg kg^{-1}) of B in *B. juncea* (L.) Czern & Coss var. Varuna and Chapka Rohini. The concentrations of B were based on the findings of Experiment 1. All the agronomic and cultural practices were kept the same as described in Experiment 1. The treatment of NaCl and/or B was given through soil. Samples were randomly collected at 45 and 60 DAS to assess various parameters and rest of the plants were harvested at about 120 DAS, to study the yield traits.

4.3.1 Shoot and root length

Shoot and root length increased as the growth progressed from 45 to 60 DAS in both the varieties, Varuna and Chapka Rohini (Table 25). However, the plants grown in the soil supplemented with NaCl (4.2 dSm^{-1}) alone or in the presence of B (20 or 60 mg kg^{-1}) significantly lost the shoot and root length at both the stages (45 and 60 DAS) of growth. Individually, B (20 mg kg^{-1}) was found to be least toxic. Moreover, the presence of NaCl (4.2 dSm^{-1}) in soil alone decreased the shoot length by 20% and 31% and root length by 26% and 34% in Varuna and Chapka Rohini, respectively, compared to their respective control plants, at 45 DAS. Furthermore, out of the two interactions, NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) generated highest toxicity and reduced the shoot length by 34% and 42% and that of root length by 41% and 49% in Varuna and Chapka Rohini, respectively, compared to their control plants, at 45 DAS. The reduction was more prominent in Chapka Rohini than Varuna at both the stages of growth.

4.3.2 Shoot and root fresh mass

The plants exposed to NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) reduced the shoot and root fresh mass in both the varieties (Varuna and Chapka rohini) at 45 and 60 DAS (Table 26). The plants raised in soil amended with NaCl (4.2 dSm^{-1}) alone significantly lost the shoot and root fresh mass by 22 % and 32% in Varuna and 35% and 38% in Chapka Rohini, respectively, compared to their control plants, at 45 DAS.

Table 25: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root length (cm) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot length						Root length					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	37.41	28.40	32.91	73.41	60.29	66.85	15.48	10.72	13.10	23.79	19.04	21.42
NaCl (4.2 dSm^{-1})	30.00	19.73	24.86	61.23	44.00	52.62	11.40	7.11	9.25	18.98	13.62	16.30
B (20 mg kg^{-1})	35.19	26.14	30.66	70.80	56.68	63.74	14.18	9.30	11.74	22.74	17.74	20.24
B (60 mg kg^{-1})	28.83	18.33	23.58	59.54	42.20	50.87	10.83	5.91	8.37	17.64	11.81	14.72
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	29.51	18.95	24.23	59.64	41.38	50.51	11.21	6.89	9.05	18.55	13.13	15.84
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	24.72	16.32	20.52	52.97	37.46	45.22	9.15	5.43	7.29	16.34	10.85	13.60
Mean	30.94	21.31		62.93	47.00		12.04	7.56		19.67	14.37	
LSD at 5%	V = 0.99 (Sig.)			V = 1.71 (Sig.)			V = 0.41 (Sig.)			V = 0.75 (Sig.)		
	T = 1.71 (Sig.)			T = 2.29 (Sig.)			T = 0.70 (Sig.)			T = 1.14 (Sig.)		
	V × T = NS			V × T = NS			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 26: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	7.78	5.89	6.84	11.26	8.54	9.90	3.28	2.79	3.04	5.31	4.85	5.08
NaCl (4.2 dSm^{-1})	6.03	3.83	4.93	9.14	6.15	7.64	2.24	1.73	1.98	3.74	3.12	3.43
B (20 mg kg^{-1})	7.28	5.32	6.30	10.75	7.90	9.32	2.83	2.21	2.52	4.93	4.35	4.64
B (60 mg kg^{-1})	5.85	3.61	4.73	8.94	5.89	7.41	2.04	1.47	1.76	3.60	2.95	3.28
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	5.87	3.72	4.80	9.01	6.00	7.50	2.17	1.67	1.92	3.68	3.05	3.36
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	5.25	3.26	4.25	8.09	5.49	6.79	1.87	1.31	1.59	3.30	2.57	2.94
Mean	6.34	4.27		9.53	6.66		2.41	1.86		4.09	3.48	
LSD at 5%	V = 0.27 (Sig.)			V = 0.37 (Sig.)			V = 0.11 (Sig.)			V = 0.21 (Sig.)		
	T = 0.48 (Sig.)			T = 0.53 (Sig.)			T = 0.14 (Sig.)			T = 0.23 (Sig.)		
	V × T = NS			V × T = NS			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Moreover, out of the two combinations of NaCl and B, the maximum decline was noted against NaCl (4.2 dSm^{-1}) + B (60 mg kg^{1}) which decreased the shoot fresh mass by 35% and 44%; root fresh mass by 43% and 53% in Varuna and Chapka Rohini, respectively, compared with their control plants, at 45 DAS.

4.3.3 Shoot and root dry mass

The data in table 27 showed that the shoot and root dry mass increased as the growth progressed from 45 to 60 DAS in both the varieties (Varuna and Chapka Rohini). However, the plants raised in the soil supplemented with NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) had lower shoot and root dry mass, as compared to the control plants. NaCl (4.2 dSm^{-1}) decreased the shoot dry mass by 26% and 38% and that of root dry mass by 32% and 40% in Varuna and Chapka Rohini, respectively, as compared to their respective control plants, at 45 DAS. Moreover, combined stress of NaCl (4.2 dSm^{-1}) and B (60 mg kg^{1}) generated maximum toxicity and reduced the shoot dry mass in Varuna and Chapka Rohini by 37% and 47% at 45 DAS and 31% and 39% at 60 DAS and that of root dry mass by 42% and 51% at 45 DAS and 37% and 45% at 60 DAS, respectively, compared to their respective control plants. Two varieties (Varuna and Chapka Rohini) showed contrasting response to stress, at both the stages of growth.

4.3.4 Leaf area

As plant age progressed from 45 to 60 day stage, per cent of the leaf area increased (Table 28). The plants exposed to NaCl (4.2 dSm^{-1}), B (20 mg kg^{-1}) or B (60 mg kg^{-1}) alone or in combination significantly lost leaf area in both the varieties (Chapka and Varuna). NaCl (4.2 dSm^{-1}) alone reduced the leaf area by 27% and 17% in Varuna and 38% and 29% in Chapka Rohini, compared to their control plants, at 45 and 60 DAS, respectively. Moreover, out of the two combinations, NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1}) and NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) tested, the later caused maximum loss in leaf area which was 36% and 27% in Varuna and 49% and 37% lower in Chapka Rohini, at 45 and 60 DAS, respectively, in comparison to their control plants. The damage was more pronounced in variety Chapka Rohini than Varuna at both the growth stages.

4.3.5 Chlorophyll content (SPAD level)

The data shown in table 28 revealed that the SPAD value of chlorophyll increased as the growth progressed from 45 to 60 day stage. However, the availability of NaCl

Table 27: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root dry mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot dry mass						Root dry mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.29	1.69	1.99	3.38	2.53	2.96	0.85	0.68	0.76	1.64	1.23	1.44
NaCl (4.2 dSm^{-1})	1.70	1.05	1.38	2.69	1.75	2.22	0.58	0.40	0.49	1.19	0.80	0.99
B (20 mg kg^{-1})	2.08	1.43	1.75	3.16	2.29	2.72	0.75	0.56	0.66	1.50	1.05	1.27
B (60 mg kg^{-1})	1.60	0.99	1.29	2.50	1.65	2.07	0.54	0.39	0.46	1.13	0.75	0.94
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	1.65	1.02	1.34	2.63	1.71	2.17	0.54	0.38	0.46	1.16	0.78	0.97
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	1.43	0.90	1.17	2.32	1.54	1.93	0.49	0.33	0.41	1.04	0.68	0.86
Mean	1.79	1.18		2.78	1.91		0.63	0.46		1.27	0.88	
LSD at 5%	V = 0.09 (Sig.)			V = 0.14 (Sig.)			V = 0.02 (Sig.)			V = 0.06 (Sig.)		
	T = 0.16 (Sig.)			T = 0.19 (Sig.)			T = 0.03 (Sig.)			T = 0.10 (Sig.)		
	V × T = 0.23 (Sig.)			V × T = 0.33 (Sig.)			V × T = 0.05 (Sig.)			V × T = 0.14 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 28: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on leaf area (cm^2) and chlorophyll content (SPAD value) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Leaf area						Chlorophyll content (SPAD value)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	34.37	24.07	29.22	43.75	32.38	38.07	44.11	29.48	36.80	57.95	38.49	48.22
NaCl (4.2 dSm^{-1})	25.06	14.83	19.95	36.30	22.99	29.65	34.59	19.80	27.19	48.16	28.16	38.16
B (20 mg kg^{-1})	31.97	20.46	26.22	41.57	30.07	35.82	42.21	27.57	34.89	55.88	36.29	46.09
B (60 mg kg^{-1})	24.15	13.86	19.01	33.70	22.11	27.90	32.68	18.60	25.64	44.71	27.01	35.86
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	24.35	14.37	19.36	35.04	22.64	28.84	34.08	19.19	26.64	47.58	27.67	37.62
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	22.00	12.37	17.19	31.83	20.38	26.11	30.11	16.84	23.47	40.83	23.68	32.25
Mean	26.98	16.66		37.03	25.10		36.30	21.91		49.18	30.22	
LSD at 5%	V = 1.01 (Sig.)			V = 1.26 (Sig.)			V = 0.17 (Sig.)			V = 0.22 (Sig.)		
	T = 1.44 (Sig.)			T = 2.09 (Sig.)			T = 0.29 (Sig.)			T = 0.38 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.41 (Sig.)			V × T = 0.54 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

(4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) in soil significantly decreased the SPAD value of chlorophyll, compared to their control plants. NaCl (4.2 dSm^{-1}) alone reduced the SPAD value of chlorophyll by 21% and 17% in Varuna and 33% and 27% in Chapka Rohini, compared to their control plants, at 45 and 60 DAS, respectively. On the other hand, B (60 mg kg^{-1}) decreased the values of SPAD chlorophyll by 26% and 37% at 45 DAS and 23% and 30% at 60 DAS in Varuna and Chapka Rohini, respectively, compared to their control plants. However, the plant exposed to NaCl (4.2 dSm^{-1}) in combination with B (60 mg kg^{-1}) had maximum reduction in SPAD value of chlorophyll which was 32% and 29% less in Varuna and 43% and 38% less in Chapka Rohini, at 45 and 60 DAS, respectively, compared to their control plants. Besides this, the loss in Chapka Rohini was more prominent than Varuna.

4.3.6 Photosynthetic parameters

The photosynthetic parameters improved as the growth progressed from 45 to 60 DAS in both the varieties (Table 29 and 30). The soil supplemented with NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) significantly decreased the net photosynthetic rate (P_N) and its related parameters [stomatal conductance (g_s), internal CO_2 concentration (C_i), transpiration rate (E)] in the leaves, compared to the control plants. Moreover, out of all the treatments tested, NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) proved most deleterious and reduced the P_N by 39% and 45%; g_s by 40% and 50%; C_i by 32% and 47%; and E by 31% and 42% at 45 DAS, whereas at 60 DAS, P_N by 30% and 38%; g_s by 32% and 43%; C_i by 26% and 37%; and E by 22% and 31% in Varuna and Chapka Rohini, respectively, as compared to unstressed (control) plants.

4.3.7 Maximum quantum yield of PS II

It is evident from the table 31 that maximum quantum yield of PSII (Fv/Fm) decreased significantly in the plant treated with NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) at both the stages (45 or 60 DAS) of growth. Moreover, the plants grown in the soil fed with NaCl (4.2 dSm^{-1}) in combination with B (60 mg kg^{-1}) generated maximum toxicity and decreased the values of Fv/Fm by 28% and 21% in Varuna and 40% and 30% in Chapka Rohini, compared to their control plant, at 45 and 60 DAS, respectively. The variety Chapka Rohini possessed less values of Fv/Fm than Varuna.

Table 29: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Net photosynthetic rate						Stomatal conductance					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	19.01	12.27	15.64	26.26	17.32	21.79	0.075	0.049	0.062	0.079	0.054	0.067
NaCl (4.2 dSm^{-1})	14.04	7.93	10.98	21.05	12.46	16.76	0.056	0.031	0.044	0.063	0.040	0.051
B (20 mg kg^{-1})	17.51	10.93	14.22	24.97	16.08	20.52	0.068	0.042	0.055	0.074	0.049	0.062
B (60 mg kg^{-1})	13.09	6.91	10.00	20.97	11.94	16.46	0.050	0.028	0.039	0.058	0.033	0.046
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	13.73	7.74	10.74	20.91	12.23	16.57	0.056	0.030	0.043	0.062	0.038	0.050
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	11.62	6.70	9.16	18.42	10.80	14.61	0.045	0.024	0.035	0.054	0.031	0.042
Mean	14.83	8.75		22.10	13.47		0.058	0.034		0.065	0.041	
LSD at 5%	V = 0.09 (Sig.)			V = 0.12 (Sig.)			V = 0.002 (Sig.)			V = 0.002 (Sig.)		
	T = 0.16 (Sig.)			T = 0.21 (Sig.)			T = 0.002 (Sig.)			T = 0.002 (Sig.)		
	V × T = 0.22 (Sig.)			V × T = 0.29 (Sig.)			V × T = 0.005 (Sig.)			V × T = 0.005 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 30: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O_2 m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Internal CO_2 concentration						Transpiration rate					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	339	256	298	371	275	323	3.47	2.56	3.02	4.66	3.44	4.05
NaCl (4.2 dSm^{-1})	261	163	212	300	205	253	2.84	1.82	2.33	4.11	2.84	3.47
B (20 mg kg^{-1})	319	234	277	357	258	307	3.33	2.37	2.85	4.49	3.26	3.87
B (60 mg kg^{-1})	248	157	203	294	192	243	2.70	1.65	2.17	3.88	2.60	3.24
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	255	158	206	293	201	247	2.86	1.81	2.33	4.07	2.80	3.43
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	230	135	182	275	173	224	2.40	1.49	1.94	3.63	2.37	3.00
Mean	275	184		315	217		2.93	1.95		4.14	2.88	
LSD at 5%	V = 1.33 (Sig.)			V = 1.64 (Sig.)			V = 0.02 (Sig.)			V = 0.02 (Sig.)		
	T = 2.31 (Sig.)			T = 2.83 (Sig.)			T = 0.03 (Sig.)			T = 0.04 (Sig.)		
	V × T = 3.26 (Sig.)			V × T = 4.01 (Sig.)			V × T = 0.05 (Sig.)			V × T = 0.05 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 31: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on maximum quantum yield of PS II and electrolyte leakage (%) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Maximum quantum yield of PS II (Fv/Fm)						Electrolyte leakage (%)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	0.840	0.708	0.774	0.975	0.829	0.902	7.10	8.05	7.58	5.82	6.70	6.26
NaCl (4.2 dSm^{-1})	0.702	0.550	0.626	0.865	0.667	0.766	8.47	9.46	8.96	6.60	7.84	7.22
B (20 mg kg^{-1})	0.789	0.634	0.711	0.931	0.766	0.848	7.57	8.84	8.20	6.10	7.21	6.65
B (60 mg kg^{-1})	0.649	0.481	0.565	0.829	0.636	0.732	8.76	10.32	9.54	6.77	8.16	7.47
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	0.681	0.538	0.609	0.849	0.656	0.753	8.54	9.62	9.08	6.66	7.93	7.29
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	0.603	0.426	0.514	0.769	0.577	0.673	9.16	10.81	9.99	7.11	8.52	7.82
Mean	0.711	0.556		0.870	0.689		8.27	9.52		6.51	7.73	
LSD at 5%	V = 0.003 (Sig.)			V = 0.006 (Sig.)			V = 0.14 (Sig.)			V = 0.10 (Sig.)		
	T = 0.006 (Sig.)			T = 0.010 (Sig.)			T = 0.28 (Sig.)			T = 0.33 (Sig.)		
	V × T = 0.008 (Sig.)			V × T = 0.014 (Sig.)			V × T = 0.29 (Sig.)			V × T = 0.21 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.3.8 Electrolyte leakage

Unlike other parameters, leaf electrolyte leakage decreased as growth advanced from 45 to 60 DAS in both Varuna and Chapka Rohini (Table 31). However, the plants raised in the soil supplemented with NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) exhibited significantly increase in electrolyte leakage at both the stages (45 or 60 DAS) of growth. Moreover, the exposure of plants with NaCl (4.2 dSm^{-1}) in combination with B (60 mg kg^{-1}) caused maximum increase being 29% and 22% in Varuna and 34% and 27% in Chapka Rohini, more as compared to their control plants, at 45 and 60 DAS, respectively.

4.3.9 Carbonic anhydrase (CA) activity

As the growth advanced from 45 to 60 days, CA activity increased (Table 32). However, the plants treated with the NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) possessed reduced activity of CA compared to untreated plants in both the varieties (Varuna and Chapka Rohini). The availability of NaCl (4.2 dSm^{-1}) alone in the soil decreased the activity of CA by 15% and 22% in Varuna and 26% and 30% in Chapka Rohini, at 45 and 60 DAS, respectively, compared to their control plants. Moreover, NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) induced a maximum loss in CA activity which was 30% (Varuna) and 38% (Chapka Rohini) lesser, at 45 DAS and 23% (Varuna) and 32% (Chapka Rohini) lesser, at 60 DAS, in comparison to their respective control plants. The losses in Chapka Rohini were more prominent than Varuna.

4.3.10 Nitrate reductase (NR) activity

Activity of NR improved as the growth progressed from 45 to 60 day stage (Table 32). However, the plants raised in soil administered with NaCl (4.2 dSm^{-1}) alone or in the presence of B (20 or 60 mg kg^{-1}) had significantly lower rate of activity of NR in both the varieties (Varuna and Chapka Rohini). Moreover, the plants grown in the soil supplemented with NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) suffered maximum toxicity where NR activity decreased by 32% and 40% at 45 DAS and 29% and 37% at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to their control plants. The decline in NR activity was more prominent in Chapka Rohini than Varuna.

4.3.11 Activity of antioxidant enzymes

The data presented in tables 33 and 34 showed that the activity of antioxidant enzymes viz. catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) increased with the age (45 to 60 DAS) of the plants (Tables 33 and 34). Moreover, the

Table 32: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and nitrate reductase (n mole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Carbonic anhydrase activity						Nitrate reductase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.46	1.73	2.10	2.43	2.06	2.25	434	335	385	572	438	505
NaCl (4.2 dSm^{-1})	2.08	1.28	1.68	2.10	1.61	1.86	330	231	280	458	308	383
B (20 mg kg^{-1})	2.35	1.62	1.99	2.37	1.96	2.16	412	307	360	552	414	483
B (60 mg kg^{-1})	1.92	1.21	1.57	1.98	1.51	1.74	317	214	266	441	299	370
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	2.05	1.24	1.64	2.07	1.57	1.82	323	227	275	446	300	373
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	1.72	1.07	1.39	1.87	1.40	1.63	295	200	248	405	277	341
Mean	2.10	1.36		2.14	1.68		352	252		479	339	
LSD at 5%	V = 0.06 (Sig.)			V = 0.05 (Sig.)			V = 11.01 (Sig.)			V = 13.35 (Sig.)		
	T = 0.07 (Sig.)			T = 0.08 (Sig.)			T = 14.65 (Sig.)			T = 16.05 (Sig.)		
	V × T = 0.14 (Sig.)			V × T = 0.12 (Sig.)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

peroxidase (units g^{-1} FM) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Catalase activity						Peroxidase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	ChapkaRohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	ChapkaRohini	Mean
Control	417	335	376	449	345	397	12.38	8.89	10.64	16.12	11.25	13.69
NaCl (4.2 dSm^{-1})	546	405	476	571	401	486	17.50	11.43	14.46	21.35	13.19	17.27
B (20 mg kg^{-1})	456	355	406	481	361	421	13.50	9.66	11.58	17.53	12.06	14.79
B (60 mg kg^{-1})	578	416	497	585	413	499	17.96	11.81	14.88	22.18	13.51	17.85
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	555	413	484	586	414	500	18.55	12.13	15.34	23.06	13.98	18.52
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	619	462	541	630	457	543	19.30	12.70	16.00	23.23	15.09	19.16
Mean	528	398		550	398		16.53	11.10		20.58	13.18	
LSD at 5%	V = 17.32 (Sig.)			V = 12.58 (Sig.)			V = 0.40 (Sig.)			V = 0.47 (Sig.)		
	T = 21.01 (Sig.)			T = 22.09 (Sig.)			T = 0.46 (Sig.)			T = 0.61 (Sig.)		
	V × T = NS			V × T = 30.81 (Sig.)			V × T = 1.22 (Sig.)			V × T = 1.14 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 34: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on superoxide dismutase (units g^{-1} FM) activity and proline content (µmol g^{-1} FM) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Superoxide dismutase activity						Proline content					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	128	110	119	155	139	147	15.40	12.27	13.84	19.42	16.13	17.78
NaCl (4.2 dSm^{-1})	190	145	168	208	172	190	24.35	17.56	20.95	29.47	21.16	25.32
B (20 mg kg^{-1})	144	121	133	169	151	160	17.36	13.56	15.46	21.14	17.19	19.17
B (60 mg kg^{-1})	196	152	174	221	182	201	25.62	18.63	22.13	30.63	22.12	26.38
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	201	150	176	217	178	198	25.24	18.15	21.70	30.36	22.04	26.20
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	218	170	194	236	194	215	27.18	20.30	23.74	33.08	24.21	28.65
Mean	180	141		201	169		22.53	16.75		27.35	20.48	
LSD at 5%	V = 6.13 (Sig.)			V = 6.38 (Sig.)			V = 0.59 (Sig.)			V = 0.79 (Sig.)		
	T = 7.00 (Sig.)			T = 9.31 (Sig.)			T = 1.03 (Sig.)			T = 1.10 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.45 (Sig.)			V × T = 1.94 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

activity of these enzymes increased further in the presence of NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}). The maximum activity of these enzyme was noted in Varuna, grown in the soil amended with NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) and the activity of CAT enzyme increased by 48%, POX by 56%, and SOD by 70% as compared to their unstressed (control) plants, at 45 DAS. The activity of all the three enzymes was lower in Chapka Rohini than Varuna, at both the stages (45 and 60 DAS) of growth.

4.3.12 Proline content

Like that of antioxidant enzymes (4.3.11), proline level also reflected a similar trend. Leaf proline content increased in both the varieties raised in soil amended with NaCl (4.2 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}), over the control plants (Table 34). Out of the two varieties, Varuna possessed higher proline content than Chapka Rohini. Maximum values for proline content in both the varieties were noted in the plants exposed to combined stress of NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}), per cent increase being 76% and 65% in Varuna and 70% and 50% in Chapka Rohini, at 45 and 60 DAS, respectively, compared to their control plants.

4.3.13 Yield attributes

All the observed yield attributes (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) significantly decreased in response to NaCl (4.2 dSm^{-1}), B (20 mg kg^{-1}) or B (60 mg kg^{-1}) alone or in combination in both the varieties, at harvest (Tables 35 and 36). The application of NaCl (4.2 dSm^{-1}) alone reduced the number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant by 20%, 14%, 11% and 19% in Varuna and 30%, 20%, 19% and 29% in Chapka Rohini, respectively, in comparison to their control plants. Moreover, the presence of NaCl (4.2 dSm^{-1}) together with B (60 mg kg^{-1}) proved most toxic and the loss was 30%, 26%, 25% and 28% in Varuna and 40%, 34%, 33% and 38% in Chapka Rohini for the number of pods per plant, number of seed per pod, mass of 100 seeds and seed yield per plant, respectively, compared to their control plants.

4.4 EXPERIMENT 4

This experiment was performed with an aim to elucidate the effect of higher concentration (5.6 dSm^{-1}) of NaCl alone or in presence of B (20 or 60 mg kg^{-1}) in *B. juncea* (L.) Czern & Coss var. Varuna and Chapka Rohini. The concentrations of B were based on the findings of Experiment 1. All the agronomic and cultural practices remained the same as described in Experiment 1. The treatment of NaCl and/or B was

Table 35: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Number of pods per plant			Number of seeds per pod		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	219	181	200	13.20	10.09	11.65
NaCl (4.2 dSm^{-1})	174	127	151	11.38	8.06	9.72
B (20 mg kg^{-1})	206	166	186	12.41	9.23	10.82
B (60 mg kg^{-1})	167	121	144	10.35	7.33	8.84
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	171	125	148	11.32	7.88	9.60
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	153	108	130	9.77	6.67	8.22
Mean	182	138		11.41	8.21	
LSD at 5%	V = 6.90 (Sig.)			V = 0.22 (Sig.)		
	T = 11.94 (Sig.)			T = 0.30 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 36: Effect of soil applied sodium chloride (NaCl; 4.2 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on 100 seed mass (mg) and seed yield per plant (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	100 seed mass			Yield per plant		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	311	264	288	7.34	5.67	6.51
NaCl (4.2 dSm^{-1})	276	212	244	5.92	4.05	4.98
B (20 mg kg^{-1})	292	228	260	6.97	5.12	6.04
B (60 mg kg^{-1})	262	199	230	5.90	3.97	4.94
NaCl (4.2 dSm^{-1}) + B (20 mg kg^{-1})	246	186	216	5.81	3.98	4.89
NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1})	234	177	205	5.30	3.51	4.41
Mean	270	211		6.21	4.38	
LSD at 5%	V = 11.30 (Sig.)			V = 0.31 (Sig.)		
	T = 10.05 (Sig.)			T = 0.45 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

applied through soil, prior to seed sowing. Samples were randomly collected at 45 and 60 DAS to assess various parameters and rest of the plants were allowed to grow up to maturity and were harvested at about 120 DAS, to study the yield attributes.

4.4.1 Shoot and root length

Shoot and root length increased with the advancement of growth from 45 to 60 DAS (Table 37). The plants grown in the soil fed with NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) had significantly lesser values for shoot and root length as compared to their control in both the varieties (Varuna and Chapka Rohini) at the two stages of growth. Moreover, NaCl (5.6 dSm^{-1}) mediated reduction was more toxic than B treatments and decreased the shoot length in Varuna and Chapka Rohini by 27% and 40% and root length by 21% and 29%, compared to their control plants, at 60 DAS. Furthermore, the combination of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{1}) generated maximum toxicity and reduced the shoot length by 42% and 54% at 45 DAS and 35% and 45% at 60 DAS; root length by 48% and 57% at 45 DAS and 39% and 51% at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to their respective control plants.

4.4.2 Shoot and root fresh mass

The data depicted in table 38 indicated that fresh mass of shoot and root increased as the growth progressed from 45 to 60 DAS in both varieties (Varuna and Chapka Rohini). The soil applied NaCl (5.6 dSm^{-1}) and B (20 or 60 mg kg^{-1}) alone or in combination generated stress that significantly decreased the shoot and root fresh mass at both the stages (45 and 60 DAS) of growth. The treatment of NaCl (5.6 dSm^{-1}) triggered a significant decrease in shoot and root fresh mass that was 27% and 36% less in Varuna and 38% and 46% less in Chapka Rohini, respectively, than their control plants, at 60 DAS. On the other hand, B (60 mg kg^{-1}) also reduced the fresh mass of shoot and root by 21% and 27% in Varuna and 31% and 39% in Chapka Rohini, respectively, compared to their control plants, at 60 DAS. However, combined stress of NaCl (5.6 dSm^{-1}) and B (60 mg kg^{-1}) induced maximum reduction in shoot fresh mass, decreasing it by 42% and 50% at 45 DAS and 32% and 43% at 60 DAS; root fresh mass by 48% and 57% at 45 DAS and 43% and 51% at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to their control plants. Among the two varieties, Varuna was more tolerant to stress than Chapka Rohini.

Table 37: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root length (cm) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot length						Root length					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	38.91	29.55	34.23	75.41	63.29	69.35	15.26	10.45	12.86	23.31	18.52	20.92
NaCl (5.6 dSm^{-1})	26.14	15.95	21.04	57.45	40.51	48.98	9.47	5.31	7.39	16.13	10.47	13.30
B (20 mg kg^{-1})	36.51	27.09	31.80	71.79	58.90	65.35	13.78	8.96	11.37	22.13	17.12	19.62
B (60 mg kg^{-1})	29.97	18.39	24.18	62.43	44.27	53.35	10.67	5.82	8.25	17.63	11.62	14.62
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	27.02	16.19	21.61	56.30	39.08	47.69	9.35	5.03	7.19	16.00	10.32	13.16
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	23.43	13.67	18.55	51.78	34.84	43.31	8.01	4.49	6.25	14.60	9.11	11.85
Mean	30.33	20.14		62.53	46.81		11.09	6.68		18.30	12.86	
LSD at 5%	V = 1.08 (Sig.)			V = 1.51 (Sig.)			V = 0.50 (Sig.)			V = 0.68 (Sig.)		
	T = 1.86 (Sig.)			T = 2.61 (Sig.)			T = 0.80 (Sig.)			T = 1.19 (Sig.)		
	V × T = NS			V × T = NS			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 38: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	7.80	5.93	6.87	11.40	8.52	9.96	3.05	2.85	2.95	5.25	4.94	5.10
NaCl (5.6 dSm^{-1})	5.24	3.32	4.28	8.32	5.21	6.77	1.81	1.43	1.62	3.35	2.65	3.00
B (20 mg kg^{-1})	7.21	5.30	6.25	10.74	7.83	9.29	2.67	2.31	2.49	4.93	4.45	4.69
B (60 mg kg^{-1})	5.79	3.65	4.72	8.94	5.90	7.42	2.04	1.56	1.80	3.83	3.01	3.42
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	5.12	3.26	4.19	8.17	5.09	6.63	1.79	1.38	1.58	3.29	2.61	2.95
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	4.53	2.96	3.75	7.70	4.83	6.26	1.59	1.21	1.40	2.97	2.40	2.68
Mean	5.95	4.07		9.21	6.23		2.16	1.79		3.94	3.34	
LSD at 5%	V = 0.26 (Sig.)			V = 0.46 (Sig.)			V = 0.09 (Sig.)			V = 0.19 (Sig.)		
	T = 0.43 (Sig.)			T = 0.64 (Sig.)			T = 0.15 (Sig.)			T = 0.25 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.22 (Sig.)			V × T = 0.47 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.4.3 Shoot and root dry mass

The dry mass of shoot and root followed the pattern similar to that of fresh mass (4.4.2) (Table 39). The decrease in values was more prominent in Chapka Rohini than in Varuna at both the stages (45 and 60 DAS) of growth. Individually, higher concentration of B (60 mg kg^{-1}) generated more toxicity than the lower concentration (20 mg kg^{-1}) of B but less than that of NaCl (5.6 dSm^{-1}). The presence of NaCl (5.6 dSm^{-1}) decreased the shoot dry mass by 37% and 48%; root dry mass by 41% and 50% in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 45 DAS. Moreover, the treatment, NaCl + B (60 mg kg^{-1}) proved most deleterious and decreased the shoot dry mass by 44% and 56% at 45 DAS and 38% and 49% at 60 DAS and root dry mass by 53% and 61% at 45 DAS and 45% and 56% at 60 DAS, in Varuna and Chapka Rohini, respectively, compared to their control plants.

4.4.4 Leaf area

The data presented in table 40 revealed that leaf area increased with the increase of plant age from 45 to 60 DAS. However, availability of NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) in the soil caused a significant loss in leaf area in both the varieties (Varuna and Chapka Rohini). The plants grown under NaCl (5.6 dSm^{-1}) experienced more damage as compared to either B (20 mg kg^{-1}) or B (60 mg kg^{-1}). The values in response to NaCl (5.6 dSm^{-1}) were 34% less in Varuna and 44% less in Chapka Rohini, as compared to their control plants, at 60 DAS. However, out of the two combinations tested, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) produced maximum toxicity and decreased the leaf area by 47% and 58% at 45 DAS and 43% and 51% at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to their controls.

4.4.5 Chlorophyll content (SPAD level)

The SPAD value of chlorophyll increased as the growth of plants progressed from 45 to 60 DAS (Table 40). The plants raised in the soil amended with NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) possessed significantly lower values of SPAD chlorophyll than the unstressed (control) plants. NaCl (5.6 dSm^{-1}) proved more deleterious to the plants than B (20 or 60 mg kg^{-1}) and significantly reduced the values of chlorophyll by 31% and 23% in Varuna and 45% and 36% in Chapka Rohini, compared to their respective control plants, at 45 and 60 day stage of growth, respectively. The interactive treatment of NaCl (5.6 dSm^{-1}) with B (60 mg kg^{-1}) generated maximum decrease in chlorophyll which was 42% (Varuna) and 51% (Chapka Rohini) less at 45

Table 39: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root dry mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot dry mass						Root dry mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.38	1.56	1.97	3.29	2.48	2.89	0.89	0.75	0.82	1.55	1.15	1.35
NaCl (5.6 dSm^{-1})	1.51	0.80	1.15	2.26	1.44	1.85	0.53	0.38	0.45	0.98	0.62	0.80
B (20 mg kg^{-1})	2.16	1.31	1.74	3.08	2.24	2.66	0.79	0.63	0.71	1.41	0.98	1.20
B (60 mg kg^{-1})	1.66	0.92	1.29	2.44	1.62	2.03	0.57	0.44	0.50	1.10	0.72	0.91
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	1.46	0.76	1.11	2.23	1.41	1.82	0.50	0.36	0.43	0.96	0.60	0.78
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	1.34	0.68	1.01	2.05	1.27	1.66	0.42	0.29	0.36	0.85	0.50	0.67
Mean	1.75	1.01		2.56	1.74		0.62	0.47		1.14	0.76	
LSD at 5%	V = 0.08 (Sig.)			V = 0.09 (Sig.)			V = 0.02 (Sig.)			V = 0.04 (Sig.)		
	T = 0.09 (Sig.)			T = 0.15 (Sig.)			T = 0.04 (Sig.)			T = 0.08 (Sig.)		
	V × T = 0.19 (Sig.)			V × T = 0.22 (Sig.)			V × T = 0.05 (Sig.)			V × T = 0.11 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 40: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on leaf area (cm^2) and chlorophyll content (SPAD value) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Leaf area						Chlorophyll content (SPAD value)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	35.63	23.46	29.55	45.01	30.21	37.61	42.28	31.24	36.76	55.32	35.89	45.61
NaCl (5.6 dSm^{-1})	20.99	11.55	16.27	29.71	16.83	23.27	28.98	17.09	23.04	42.54	22.99	32.76
B (20 mg kg^{-1})	33.31	20.29	26.80	42.81	27.26	35.03	40.23	29.37	34.80	53.27	33.96	43.61
B (60 mg kg^{-1})	25.49	13.88	19.69	34.89	20.75	27.82	31.32	19.93	25.63	42.83	25.38	34.11
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	20.37	11.09	15.73	28.65	16.54	22.60	28.40	16.64	22.52	41.86	22.81	32.33
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	18.88	9.84	14.36	25.75	14.81	20.28	24.43	15.31	19.87	37.84	19.60	28.72
Mean	25.78	15.02		34.47	21.07		32.61	21.60		45.61	26.77	
LSD at 5%	V = 1.04 (Sig.)			V = 1.38 (Sig.)			V = 0.13 (Sig.)			V = 0.19 (Sig.)		
	T = 1.31 (Sig.)			T = 2.30 (Sig.)			T = 1.04 (Sig.)			T = 0.49 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.32 (Sig.)			V × T = 0.47 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

DAS and 32% (Varuna) and 45% (Chapka Rohini) less, at 60 DAS, as compared to their control plants. Moreover, Varuna possessed higher level of SPAD chlorophyll than Chapka Rohini.

4.4.6 Net photosynthetic rate and related attributes

The photosynthetic attributes [net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E)] significantly decreased in the plants grown in the presence of NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) (Tables 41 and 42). Individually, NaCl (5.6 dSm^{-1}) was more toxic than B and reduced the P_N, g_s, C_i, and E by 37%, 50%, 33% and 29% in Varuna and 50%, 55%, 46% and 47% in Chapka Rohini, respectively, at 45 DAS. However, the maximum reduction in all the aforesaid attributes was noted in the plants grown in soil amended with NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) which decreased P_N by 47% and 38%; g_s by 49% and 40%; C_i by 41% and 34%; and E by 37% and 30% in Varuna whereas, the same treatment decreased the P_N by 53% and 48%; g_s by 56% and 49%; C_i by 53% and 46%; and E by 48% and 40% in Chapka Rohini, compared to their respective control plants, at 45 and 60 DAS, respectively. Out of the two varieties, Varuna had higher photosynthetic efficiency than Chapka Rohini in control/ stress.

4.4.7 Maximum quantum yield of PS II (Fv/Fm)

It is evident from the table 43 that the maximum quantum yield of PS II (Fv/Fm) increased as the growth progressed from 45 to 60 days. However, the Fv/Fm significantly decreased in the plants that recieved NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) in both varieties (Varuna and Chapka Rohini). Individually, NaCl (5.6 dSm^{-1}) proved injurious as compared to B and decreased Fv/Fm by 25% and 21% in Varuna and 37% and 27% in Chapka Rohini, compared to their unstressed (control) plants, at 45 and 60 DAS, respectively. Among two combinations of NaCl and B tested, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) invoked a higher decrease in Fv/Fm which was 32% and 26% lower in Varuna and 43% and 35% lower in Chapka Rohini, at 45 and 60 DAS, respectively, compared to their control plants. Varuna was more efficient in PSII activity than Chapka Rohini.

4.4.8 Electrolyte leakage

The electrolyte leakage decreased as the growth progressed from 45 to 60 DAS (Table 43). However, the plants raised in the soil amended with NaCl (5.6 dSm^{-1}) and/ or B (20 or 60 mg kg^{-1}) possessed significantly higher leakage of electrolyte than

Table 41: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Net photosynthetic rate						Stomatal conductance					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	19.30	12.50	15.92	26.10	17.51	21.81	0.070	0.043	0.057	0.086	0.057	0.072
NaCl (5.6 dSm^{-1})	12.17	6.49	9.33	17.75	10.80	14.27	0.042	0.022	0.032	0.060	0.033	0.046
B (20 mg kg^{-1})	17.96	11.19	14.57	24.97	16.18	20.57	0.063	0.037	0.050	0.081	0.052	0.067
B (60 mg kg^{-1})	13.30	7.46	10.38	20.10	12.11	16.11	0.047	0.025	0.036	0.064	0.036	0.050
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	11.83	6.28	9.06	17.46	10.64	14.05	0.041	0.022	0.031	0.058	0.032	0.045
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	10.27	5.87	8.07	16.07	9.17	12.62	0.036	0.019	0.027	0.051	0.029	0.040
Mean	14.14	8.30		20.41	12.73		0.050	0.028		0.067	0.040	
LSD at 5%	V = 0.09 (Sig.)			V = 0.15 (Sig.)			V = 0.002 (Sig.)			V = 0.003 (Sig.)		
	T = 0.83 (Sig.)			T = 0.26 (Sig.)			T = 0.003 (Sig.)			T = 0.004 (Sig.)		
	V × T = 0.23 (Sig.)			V × T = 0.36 (Sig.)			V × T = 0.005 (Sig.)			V × T = 0.007 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 42: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O_2 m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Internal CO_2 concentration						Transpiration rate					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	345	250	298	376	284	330	3.42	2.43	2.93	4.59	3.39	3.99
NaCl (5.6 dSm^{-1})	231	134	183	275	171	223	2.40	1.51	1.96	3.64	2.40	3.02
B (20 mg kg^{-1})	325	228	276	362	264	313	3.28	2.25	2.77	4.43	3.19	3.81
B (60 mg kg^{-1})	258	150	204	302	193	247	2.73	1.63	2.18	3.85	2.61	3.23
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	225	131	178	273	170	221	2.36	1.46	1.91	3.52	2.33	2.92
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	202	119	160	248	152	200	2.16	1.26	1.71	3.23	2.03	2.63
Mean	264	169		306	206		2.73	1.76		3.88	2.66	
LSD at 5%	V = 0.95 (Sig.)			V = 1.66 (Sig.)			V = 0.02 (Sig.)			V = 0.02 (Sig.)		
	T = 1.65 (Sig.)			T = 2.88 (Sig.)			T = 0.03 (Sig.)			T = 0.04 (Sig.)		
	V × T = 2.33 (Sig.)			V × T = 4.07 (Sig.)			V × T = 0.04 (Sig.)			V × T = 0.06 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 43: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on maximum quantum yield of PS II and electrolyte leakage (%) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Maximum quantum yield of PS II (Fv/Fm)						Electrolyte leakage (%)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	0.836	0.703	0.770	0.969	0.837	0.903	7.10	8.05	7.58	5.82	6.70	6.26
NaCl (5.6 dSm^{-1})	0.625	0.442	0.534	0.763	0.607	0.685	9.00	10.65	9.82	7.00	8.44	7.72
B (20 mg kg^{-1})	0.785	0.632	0.709	0.921	0.775	0.848	7.57	8.84	8.20	6.10	7.21	6.65
B (60 mg kg^{-1})	0.663	0.491	0.577	0.809	0.604	0.707	8.76	10.32	9.54	6.77	8.16	7.47
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	0.612	0.431	0.521	0.753	0.601	0.677	9.08	10.85	9.96	7.18	8.46	7.82
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	0.567	0.400	0.483	0.714	0.541	0.627	9.83	11.67	10.75	7.64	9.36	8.50
Mean	0.681	0.516		0.822	0.661		8.56	10.06		6.75	8.05	
LSD at 5%	V = 0.003 (Sig.)			V = 0.005 (Sig.)			V = 0.08 (Sig.)			V = 0.05 (Sig.)		
	T = 0.005 (Sig.)			T = 0.009 (Sig.)			T = 0.25 (Sig.)			T = 0.22 (Sig.)		
	V × T = 0.007 (Sig.)			V × T = 0.012 (Sig.)			V × T = 0.19 (Sig.)			V × T = 0.13 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

the unstressed (control) plants. The plants exposed to NaCl (5.6 dSm^{-1}) had more leakage of electrolytes than B (20 or 60 mg kg^{-1}) at both the stages of growth. The stress generated by the interaction of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) was most prominent and triggered maximum increase in the values which were 38% (Varuna) and 45% (Chapka Rohini) at 45 DAS and 31% (Varuna) and 40% (Chapka Rohini) more at 60 DAS, compared to their control plants. Moreover, Chapka Rohini showed more electrolyte leakage than Varuna

4.4.9 Carbonic anhydrase (CA) activity

The data presented in the table 44 indicated that CA activity improved as the growth progressed from 45 to 60 DAS. However, the plants grown in the soil supplemented with NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) had lesser activity of CA in both the varieties (Varuna and Chapka rohini) as compared to control plants. The plants exposed to NaCl (5.6 dSm^{-1}) reduced the activity of CA by 22% and 30% in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 60 DAS. Furthermore, the plants raised in soil fed with NaCl (5.6 dSm^{-1}) in combination with B (60 mg kg^{-1}) generated maximum damage where loss in CA activity was 37% and 30% in Varuna and 48% and 41% in Chapka Rohini, at 45 and 60 DAS, respectively, compared with their control plants. Among the two varieties, Chapka Rohini was more sensitive to NaCl and/or B stress than Varuna.

4.4.10 Nitrate reductase (NR) activity

The activity of NR was higher at later stage (60 DAS) than at an early stage (45 DAS) of growth (Table 44). However, there was a significant decline in the activity of NR in response to NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) as compared to control plants. Individually, NaCl (5.6 dSm^{-1}) triggered more damage compared to B (20 or 60 mg kg^{-1}) and decreased the NR activity by 33% and 42% in Varuna and 26% and 39% in Chapka Rohini, compared to their non-stressed (control) plants, at 45 and 60 DAS, respectively. However, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) triggered maximum decrease of 36% and 42% at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to their control plants. However, the activity of NR was higher in Varuna than Chapka Rohini, at both the stages (45 and 60 DAS) of growth.

4.4.11 Antioxidant enzymes activity

The activity of antioxidative enzymes i.e., catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) increased as the growth progressed from 45 to 60 DAS

Table 44: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and nitrate reductase (nmole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Carbonic anhydrase activity						Nitrate reductase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.20	1.70	1.95	2.39	2.00	2.20	429	329	379	569	433	501
NaCl (5.6 dSm^{-1})	1.63	1.10	1.36	1.85	1.40	1.62	289	192	241	420	266	343
B (20 mg kg^{-1})	2.11	1.59	1.85	2.33	1.89	2.11	406	301	353	546	410	478
B (60 mg kg^{-1})	1.74	1.20	1.47	1.94	1.47	1.71	312	212	262	432	291	361
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	1.59	1.08	1.33	1.83	1.38	1.60	284	185	235	408	259	333
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	1.38	0.88	1.13	1.66	1.18	1.42	251	167	209	363	233	298
Mean	1.77	1.26		2.00	1.55		329	231		456	315	
LSD at 5%	V = 0.04 (Sig.)			V = 0.04 (Sig.)			V = 12.08 (Sig.)			V = 13.68 (Sig.)		
	T = 0.06 (Sig.)			T = 0.07 (Sig.)			T = 19.93 (Sig.)			T = 15.69 (Sig.)		
	V × T = 0.11 (Sig.)			V × T = 0.09 (Sig.)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

in both the varieties i.e. Varuna and Chapka Rohini (Tables 45 and 46). Moreover, the application of NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) to the soil induced the activity of these enzymes. NaCl (5.6 dSm^{-1}) in combination with B (60 mg kg^{-1}) generated maximum increase in CAT (58%), POX (66%) and SOD (96%) in Varuna, compared to their control plants, at 45 DAS. Control (stress-free) plants possessed minimum activity of all these antioxidant enzymes. Out of the two varieties, Chapka Rohini possessed lower activity of antioxidant enzymes than Varuna, at both stages (45 and 60 DAS) of growth.

4.4.12 Proline content

The level of proline exhibited an increase in the plants which were exposed to NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) stress (Table 46). The maximum accumulation of proline was noted in the plants that were raised in the soil amended with NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) in both the varieties. In terms of percentage, the maximum increase was 90% and 82% in Varuna and 72% and 60% in Chapka Rohini, over their control plants, at 45 and 60 DAS, respectively. Chapka Rohini had lower values than Varuna at both the stages of growth. The control plants showed the least values for the proline content.

4.4.13 Yield characteristics

The yield characteristics (number of pods per plant, number of seeds per pod, mass of 100 seeds and seed yield per plant) significantly decreased in the plants exposed to 5.6 dSm^{-1} of NaCl alone or in association with B (20 or 60 mg kg^{-1}) in both the varieties i.e. Varuna and Chapka Rohini (Tables 47 and 48). NaCl (5.6 dSm^{-1}) was more toxic than B (20 or 60 mg kg^{-1}) alone and reduced the number of pods per plant, number of seeds per pod, mass of 100 seeds and seed yield per plant by 30%, 28%, 23% and 29% in Varuna and 40%, 35%, 32% and 38% in Chapka Rohini, respectively, at harvest. However, the simultaneous stress of NaCl (5.6 dSm^{-1}) with B (60 mg kg^{-1}) generated severe toxicity and decreased the number of pods by 39% and 49%; number of seeds by 33% and 42%; mass of 100 seeds by 34% and 40%; and seed yield by 35% and 47% in Varuna and Chapka Rohini, respectively, compared to their stress free (control) plants, at harvest.

Table 45: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on catalase (mM H_2O_2 decomposed g^{-1} FM) and peroxidase (units g^{-1} FM) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Catalase activity						Peroxidase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	411	331	371	445	340	393	11.99	8.85	10.42	16.04	11.17	13.61
NaCl (5.6 dSm^{-1})	573	426	499	582	426	504	17.87	12.23	15.05	22.74	13.91	18.32
B (20 mg kg^{-1})	449	354	401	477	357	417	13.16	9.59	11.37	17.47	12.04	14.75
B (60 mg kg^{-1})	554	413	484	553	405	479	16.96	11.47	14.21	21.95	13.73	17.84
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	587	436	512	598	439	519	18.44	12.81	15.63	23.31	14.55	18.93
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	650	488	569	665	478	572	19.87	13.57	16.72	24.57	15.79	20.18
Mean	537	408		553	408		16.38	11.42		21.01	13.53	
LSD at 5%	V = 12.84 (Sig.)			V = 13.55 (Sig.)			V = 0.53 (Sig.)			V = 0.46 (Sig.)		
	T = 19.34 (Sig.)			T = 22.19 (Sig.)			T = 0.92 (Sig.)			T = 0.80 (Sig.)		
	V × T = NS			V × T = 33.19 (Sig.)			V × T = 1.30 (Sig.)			V × T = 1.14 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 46: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on superoxide dismutase (units g^{-1} FM) activity and proline content (µmol g^{-1} FM) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Superoxide dismutase activity						Proline content					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	125	103	114	152	136	144	15.32	12.21	13.77	19.29	16.05	17.67
NaCl (5.6 dSm^{-1})	205	161	183	235	194	214	26.06	19.21	22.64	32.25	22.95	27.60
B (20 mg kg^{-1})	141	114	127	167	147	157	17.26	13.53	15.39	21.00	17.15	19.08
B (60 mg kg^{-1})	192	142	167	216	177	197	25.10	18.32	21.71	30.51	22.00	26.26
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	212	166	189	241	200	220	27.10	20.02	23.56	32.86	23.86	28.36
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	245	194	220	274	231	253	29.11	21.06	25.09	35.11	25.74	30.43
Mean	187	147		214	181		23.32	17.39		28.50	21.29	
LSD at 5%	V = 4.26 (Sig.)			V = 5.78 (Sig.)			V = 0.90 (Sig.)			V = 0.85 (Sig.)		
	T = 7.37 (Sig.)			T = 10.02 (Sig.)			T = 1.08 (Sig.)			T = 1.29 (Sig.)		
	V × T = NS			V × T = NS			V × T = 2.20 (Sig.)			V × T = 2.07 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 47: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Number of pods per plant			Number of seeds per pod		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	215	178	197	13.18	10.03	11.61
NaCl (5.6 dSm^{-1})	151	107	129	9.53	6.46	7.99
B (20 mg kg^{-1})	203	163	183	12.46	9.23	10.84
B (60 mg kg^{-1})	160	119	139	10.27	7.22	8.74
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	149	105	127	9.42	6.32	7.87
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	132	90	111	8.78	5.83	7.30
Mean	168	127		10.61	7.51	
LSD at 5%	V = 6.29 (Sig.)			V = 0.63 (Sig.)		
	T = 9.40 (Sig.)			T = 0.54 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 48: Effect of soil applied sodium chloride (NaCl; 5.6 dSm^{-1}) and/or boron (B; 20 or 60 mg kg^{-1} soil) on 100 seed mass (mg) and seed yield per plant (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	100 seed mass			Yield per plant		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	309	268	289	7.21	5.58	6.40
NaCl (5.6 dSm^{-1})	238	182	210	5.12	3.47	4.29
B (20 mg kg^{-1})	289	233	261	6.84	4.97	5.90
B (60 mg kg^{-1})	261	203	232	5.82	3.98	4.90
NaCl (5.6 dSm^{-1}) + B (20 mg kg^{-1})	235	180	208	5.02	3.41	4.22
NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})	205	162	183	4.68	2.98	3.83
Mean	256	205		5.78	4.07	
LSD at 5%	V = 10.73 (Sig.)			V = 0.23 (Sig.)		
	T = 18.59 (Sig.)			T = 0.40 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.5 EXPERIMENT 5

This experiment was conducted to dissect the role of exogenously sourced two analogues (28-homobrassinolide or 24-epibrassinolide) of brassinosteroid under different levels of B in two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. All the agronomic and cultural practices were kept the same as described in Experiment 1. The treatment of B (20 mg kg^{-1} or 60 mg kg^{-1}) was given through soil before sowing as stated in earlier experiments and the foliage of 44 day old plants was sprayed with DDW or 10^{-8} M of HBL or EBL. Samples were randomly collected at 45 and 60 DAS to study various parameters and rest of the plants were harvested at about 120 DAS, to study the yield traits.

4.5.1 Shoot and root length

The length of shoot and root increased with the advancement of plant growth from 45 to 60 days in both the varieties, Varuna and Chapka Rohini (Table 49). However, the plants grown under different levels (20 or 60 mg kg^{-1} of soil) of B had shorter shoot and root at both 45 and 60 DAS, as compared to their respective controls. The highest level (60 mg kg^{-1}) of B caused maximum loss in shoot length that was 26% and 40% lesser at 45 DAS and 19% and 32% lesser at 60 DAS; whereas in root length that was 30% and 45%, lesser at 45 DAS and 24% and 38%, lesser at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to stress free control plants. Application of dilute solution of brassinosteroid analogues (10^{-8} M HBL or EBL) to the foliage of stress free 60 day old plants significantly increased the shoot and root length as compared to control plants, in both the varieties. Out of them, EBL was more effective than HBL. The maximum per cent increase in shoot and root length by EBL was 70% and 57% more in Varuna and 60% and 43% higher in Chapka Rohini, than their respective 60 day old control plants. Moreover, the stressed plants treated with HBL or EBL completely recovered from the damaging effects generated by the two levels (20 or 60 mg kg^{-1}) of the B, at 60 DAS. The response of Varuna was better than Chapka Rohini.

4.5.2 Shoot and root fresh mass

The foliar spray of HBL or EBL (10^{-8} M) significantly increased the shoot and root fresh mass in stress-free plants in both the varieties (Varuna and Chapka Rohini), at 60 DAS (Table 50). Moreover, EBL was more effective, increasing the values in shoot and root by 62% and 65% (Varuna) and 50% and 60% (Chapka Rohini), respectively, at 60 DAS, compared to their respective control plants. However, the

Table 49: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root length (cm) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot length						Root length					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	39.41	28.40	33.91	72.41	61.29	66.85	15.86	11.73	13.80	26.23	20.05	23.14
HBL (10^{-8} mM)	39.95	28.56	34.25	119.48	95.77	107.62	15.82	11.66	13.74	37.24	27.46	32.35
EBL (10^{-8} mM)	40.07	28.63	34.35	123.39	98.28	110.84	15.89	11.75	13.82	41.19	28.67	34.93
B (20 mg kg^{-1})	36.23	25.55	30.89	67.99	55.89	61.94	14.37	10.18	12.28	24.28	17.87	21.08
B (60 mg kg^{-1})	29.25	17.02	23.14	58.58	41.51	50.04	11.10	6.46	8.78	19.86	12.44	16.15
B (20 mg kg^{-1})+HBL	35.84	25.09	30.46	94.14	75.15	84.65	14.44	10.09	12.26	32.59	24.01	28.30
B (60 mg kg^{-1})+HBL	28.45	17.20	22.82	79.00	64.50	71.75	10.62	6.08	8.35	28.26	20.54	24.40
B (20 mg kg^{-1})+EBL	36.58	25.41	31.00	99.95	78.19	89.07	14.43	10.11	12.27	34.13	25.39	29.76
B (60 mg kg^{-1})+EBL	29.37	14.77	22.07	83.16	66.70	74.93	11.11	5.87	8.49	29.89	22.24	26.06
Mean	35.02	23.40		88.68	70.81		13.74	9.33		30.41	22.07	
LSD at 5%	V = 0.79 (Sig.)			V = 2.66 (Sig.)			V = 0.46 (Sig.)			V = 0.90 (Sig.)		
	T = 1.68 (Sig.)			T = 3.10 (Sig.)			T = 0.97 (Sig.)			T = 1.90 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.38 (Sig.)			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 50: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	6.78	5.51	6.15	12.72	10.00	11.36	2.96	2.47	2.72	5.49	4.88	5.19
HBL (10^{-8} mM)	6.82	5.52	6.17	18.45	14.12	16.28	2.93	2.43	2.68	8.20	7.11	7.65
EBL (10^{-8} mM)	6.81	5.53	6.17	20.66	15.00	17.83	3.01	2.47	2.74	9.06	7.80	8.43
B (20 mg kg^{-1})	6.25	4.94	5.60	12.03	9.16	10.60	2.56	1.96	2.26	5.10	4.38	4.74
B (60 mg kg^{-1})	5.16	3.42	4.29	10.05	6.89	8.47	1.84	1.19	1.52	3.73	2.97	3.35
B (20 mg kg^{-1})+HBL	6.24	4.91	5.57	16.53	11.90	14.21	2.54	1.95	2.25	7.30	6.19	6.75
B (60 mg kg^{-1})+HBL	5.02	3.42	4.22	14.00	10.66	12.33	1.80	1.16	1.48	5.81	5.03	5.42
B (20 mg kg^{-1})+EBL	6.30	5.01	5.66	17.04	12.40	14.72	2.56	1.95	2.26	7.47	6.30	6.88
B (60 mg kg^{-1})+EBL	5.22	3.53	4.37	14.61	11.00	12.81	1.83	1.20	1.52	5.98	5.14	5.56
Mean	6.07	4.64		15.12	11.24		2.45	1.87		6.46	5.53	
LSD at 5%	V = 0.18 (Sig.)			V = 0.57 (Sig.)			V = 0.10 (Sig.)			V = 0.28 (Sig.)		
	T = 0.38 (Sig.)			T = 1.20 (Sig.)			T = 0.21 (Sig.)			T = 0.60 (Sig.)		
	V × T = 0.54 (Sig.)			V × T = 1.70 (Sig.)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

plants raised in the soil supplemented with B (20 or 60 mg kg^{-1}) exhibited lower values. This damaging effect was completely overcome by the follow up treatment of stressed plants with BRs (HBL or EBL, 10^{-8} M), in both the varieties, at the 60 day stage of growth. Moreover, Varuna responded better than Chapka Rohini.

4.5.3 Shoot and root dry mass

The shoot and root dry mass followed a patter similar to that of fresh mass (4.5.2). The plants raised in the soil amended with B (20 or 60 mg kg^{-1}) had lesser dry mass (Table 51). Moreover, the decrease was more prominent at 45 day stage than at 60 day stage of growth. Exogenous application of brassinosteroid analogues (HBL/EBL; 10^{-8} M) significantly increased the shoot and root dry mass, at 60 day stage of growth. EBL proved better than HBL which increased the shoot dry mass by 76% and 68% and root dry mass by 80% and 72% in Varuna and Chapka Rohini, respectively, as compared to their respective control, at 60 DAS. The damage caused by B stress was completely nullified by BRs, at 60 DAS. Among the two varieties, Varuna was more responsive than Chapka Rohini.

4.5.4 Leaf area

Table 52 depicts that like other growth parameters, leaf area also increased with the advancement of plant age from 45 to 60 DAS. However, with the increase in the level of soil B (20 or 60 mg kg^{-1}) the leaf area decreased proportionately in both the varieties (Varuna and Chapka Rohini). BR analogues (HBL/EBL) applied to the foliage of the plants significantly increased the leaf area in stress free plants where EBL generated higher values (40% more than control) in Varuna, at 60 DAS. Moreover, toxicity generated by B was also completely neutralized by EBL in both the varieties, at 60 DAS. Moreover, variety Varuna was more responsive than Chapka Rohini, at both the stages of growth.

4.5.5 Chlorophyll content (SPAD value)

The plants at 60 day stage of growth had higher chlorophyll content (SPAD value) than at 45 day stage (Table 52). The application of brassinosteroids (HBL/EBL) to the foliage of unstressed (control) plants increased the values in both the varieties, at 60 day stage of growth. The per cent increase in chlorophyll content by HBL /EBL was 28% and 39% higher in Varuna and 21% and 27% more in Chapka Rohini, respectively, compared to their control plants, at 60 DAS. However, EBL was found more effective than HBL in both the varieties (Varuna and Chapka Rohini). Contrary

Table 51: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root dry mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot dry mass						Root dry mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	1.96	1.54	1.75	3.16	2.68	2.92	0.82	0.66	0.74	1.58	1.27	1.43
HBL (10^{-8} mM)	1.99	1.52	1.76	5.10	4.04	4.57	0.82	0.66	0.74	2.65	2.00	2.32
EBL (10^{-8} mM)	2.02	1.53	1.77	5.57	4.50	5.03	0.81	0.67	0.74	2.85	2.18	2.52
B (20 mg kg^{-1})	1.78	1.31	1.55	2.79	2.35	2.57	0.72	0.55	0.63	1.45	1.08	1.26
B (60 mg kg^{-1})	1.36	0.93	1.14	2.36	1.75	2.05	0.52	0.38	0.45	1.08	0.77	0.93
B (20 mg kg^{-1})+HBL	1.74	1.31	1.52	4.01	3.21	3.61	0.70	0.54	0.62	2.07	1.59	1.83
B (60 mg kg^{-1})+HBL	1.29	0.57	0.93	3.42	2.76	3.09	0.51	0.34	0.43	1.66	1.32	1.49
B (20 mg kg^{-1})+EBL	1.79	1.32	1.55	4.20	3.35	3.77	0.73	0.54	0.63	2.17	1.68	1.93
B (60 mg kg^{-1})+EBL	1.35	0.60	0.98	3.51	2.82	3.16	0.52	0.36	0.44	1.69	1.36	1.52
Mean	1.70	1.18		3.79	3.05		0.68	0.52		1.91	1.47	
LSD at 5%	V = 0.06 (Sig.)			V = 0.14 (Sig.)			V = 0.02 (Sig.)			V = 0.06 (Sig.)		
	T = 0.13 (Sig.)			T = 0.29 (Sig.)			T = 0.04 (Sig.)			T = 0.18 (Sig.)		
	V × T = 0.18 (Sig.)			V × T = 0.42 (Sig.)			V × T = 0.06 (Sig.)			V × T = 0.17 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 52: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on leaf area (cm^2) and chlorophyll content (SPAD value) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Leaf area						Chlorophyll content (SPAD value)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	34.17	31.62	32.90	41.65	37.32	39.49	43.00	35.79	39.40	57.74	49.50	53.62
HBL (10^{-8} mM)	34.34	31.66	33.00	53.73	45.60	49.67	43.39	36.08	39.73	73.91	60.11	67.01
EBL (10^{-8} mM)	34.43	31.78	33.10	58.73	50.01	54.37	43.47	36.15	39.81	80.26	62.87	71.56
B (20 mg kg^{-1})	31.62	27.12	29.37	38.36	34.06	36.21	41.15	33.47	37.31	55.68	46.67	51.18
B (60 mg kg^{-1})	24.00	18.51	21.26	32.03	25.21	28.62	31.85	22.59	27.22	44.55	34.73	39.64
B (20 mg kg^{-1})+HBL	31.83	26.92	29.38	50.89	43.85	47.37	41.50	33.60	37.55	66.27	54.04	60.16
B (60 mg kg^{-1})+HBL	24.26	12.64	18.45	44.86	39.52	42.19	31.65	22.49	27.07	63.91	51.94	57.92
B (20 mg kg^{-1})+EBL	32.09	26.85	29.47	54.23	45.52	49.87	41.66	33.67	37.66	68.80	57.25	63.03
B (60 mg kg^{-1})+EBL	24.43	17.38	20.91	46.27	40.35	43.31	31.79	22.75	27.27	65.74	53.68	59.71
Mean	30.13	24.94		46.75	40.16		38.83	30.73		64.10	52.31	
LSD at 5%	V = 0.84 (Sig.)			V = 1.36 (Sig.)			V = 0.12 (Sig.)			V = 0.20 (Sig.)		
	T = 1.78 (Sig.)			T = 2.90 (Sig.)			T = 0.46 (Sig.)			T = 0.58 (Sig.)		
	V × T = 2.52 (Sig.)			V × T = NS			V × T = 0.52 (Sig.)			V × T = 0.76 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

to this, B treatment (20 or 60 mg kg^{-1}) significantly lowered the chlorophyll content in both the varieties, at the two stages of growth. However, the application of BRs (HBL/EBL) as a follow up treatment completely neutralized the inhibitory effects of B stress in both the varieties, at 60 day stage of growth. Higher values of SPAD chlorophyll were noted in Varuna than Chapka Rohini.

4.5.6 Net photosynthetic rate and related attributes

Net photosynthetic rate (P_N) and its related attributes i.e. stomatal conductance (g_s), internal CO_2 concentrations (C_i), and transpiration rate (E) improved as the growth advanced from 45 to 60 day stage of growth (Tables 53 and 54). However, the values decreased in the presence of B (20 or 60 mg kg^{-1}) in a concentration dependent manner. However, the leaves of unstressed plants exposed to either of the BR analogues (10^{-8} M HBL/EBL) had higher values of all the aforesaid parameters in both the varieties, at 60 DAS. The maximum values for P_N, g_s, C_i and E were noted by the application of EBL alone that were 42%, 70%, 46% and 37% higher in Varuna and 31%, 58%, 35% and 29% more in Chapka Rohini, compared to their control plants, respectively, at 60 day stage of growth. Besides this, the foliage of stressed plants sprayed with either of the BR analogues completely nullified the damaging effects of B, at 60 day stage of growth. Out of the two varieties, Chapka Rohini was more vulnerable to stress than Varuna.

4.5.7 Maximum quantum yield of PSII

The values of Fv/Fm increased as the growth of plant advanced from 45 to 60 DAS. The leaves of the plants sprayed with either of the brassinosteroid analogues (HBL/EBL) had higher values for Fv/Fm, compared to their control plants. However, application of B as soil amendment caused significant reduction in Fv/Fm values in both the varieties (Varuna and Chapka Rohini) (Table 55). The higher concentration (60 mg kg^{-1}) of B was more toxic decreasing the values by 24% and 37% at 45 DAS and 15% and 26% at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to the corresponding stress free control plants. This damage was completely overcome by the application of either of the BRs to the foliage of stressed plants, at 60 DAS. Varuna showed better photosynthetic responses to the treatment than Chapka Rohini.

Table 53: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Net photosynthetic rate						Stomatal conductance					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	19.19	14.56	16.88	28.60	18.68	23.64	0.069	0.051	0.060	0.084	0.068	0.076
HBL (10^{-8} mM)	20.90	15.27	18.08	37.01	22.79	29.90	0.078	0.055	0.066	0.129	0.096	0.113
EBL (10^{-8} mM)	22.11	15.92	19.02	40.71	24.56	32.63	0.083	0.058	0.070	0.143	0.108	0.125
B (20 mg kg^{-1})	17.28	12.68	14.98	26.85	16.86	21.86	0.061	0.044	0.053	0.080	0.062	0.071
B (60 mg kg^{-1})	13.23	8.62	10.93	22.08	12.43	17.26	0.044	0.029	0.037	0.061	0.041	0.051
B (20 mg kg^{-1})+HBL	19.95	14.70	17.32	35.09	21.68	28.38	0.072	0.052	0.062	0.108	0.078	0.093
B (60 mg kg^{-1})+HBL	13.42	8.67	11.05	31.48	19.80	25.64	0.046	0.029	0.038	0.092	0.073	0.082
B (20 mg kg^{-1})+EBL	21.01	15.42	18.22	37.13	22.60	29.86	0.074	0.053	0.064	0.111	0.079	0.095
B (60 mg kg^{-1})+EBL	17.26	10.08	13.67	33.48	21.07	27.28	0.049	0.030	0.040	0.099	0.077	0.088
Mean	18.26	12.88	16.88	32.49	20.05		0.064	0.045		0.101	0.076	
LSD at 5%	V = 0.09 (Sig.)			V = 0.12 (Sig.)			V = 0.002 (Sig.)			V = 0.002 (Sig.)		
	T = 0.32 (Sig.)			T = 0.79 (Sig.)			T = 0.004 (Sig.)			T = 0.006 (Sig.)		
	V × T = 0.46 (Sig.)			V × T = 0.85 (Sig.)			V × T = 0.007 (Sig.)			V × T = 0.005 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 54: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1}) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O_2 m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Internal CO_2 concentration						Transpiration rate					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	331	264	298	377	348	363	3.39	3.18	3.29	4.88	4.34	4.61
HBL (10^{-8} mM)	385	290	328	498	441	469	3.71	3.40	3.55	6.05	5.21	5.63
EBL (10^{-8} mM)	413	314	353	550	471	511	3.96	3.56	3.76	6.69	5.60	6.15
B (20 mg kg^{-1})	312	241	276	362	326	344	3.25	2.96	3.10	4.71	4.09	4.40
B (60 mg kg^{-1})	246	157	201	305	235	270	2.65	2.07	2.36	4.14	3.10	3.62
B (20 mg kg^{-1})+HBL	334	266	300	447	382	414	3.41	3.19	3.30	5.68	4.77	5.22
B (60 mg kg^{-1})+HBL	248	158	203	380	350	365	2.69	2.36	2.52	5.36	4.55	4.96
B (20 mg kg^{-1})+EBL	337	268	302	450	384	417	3.43	3.21	3.32	6.02	4.99	5.51
B (60 mg kg^{-1})+EBL	248	159	204	388	352	370	2.72	2.71	2.71	5.61	4.73	5.17
Mean	313	235		417	365		3.24	2.96		5.46	4.60	
LSD at 5%	V = 0.80 (Sig.)			V = 1.07 (Sig.)			V = 0.02 (Sig.)			V = 0.02 (Sig.)		
	T = 1.69 (Sig.)			T = 2.86 (Sig.)			T = 0.04 (Sig.)			T = 0.05 (Sig.)		
	V × T = 2.39 (Sig.)			V × T = 3.20 (Sig.)			V × T = 0.05 (Sig.)			V × T = 0.05 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 55: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on maximum quantum yield of PS II and electrolyte leakage (%) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Maximum quantum yield of PS II (Fv/Fm)						Electrolyte leakage					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	0.772	0.745	0.759	0.802	0.765	0.784	7.16	8.20	7.68	5.80	6.78	6.29
HBL (10^{-8} mM)	0.802	0.767	0.784	0.923	0.841	0.882	7.16	8.22	7.69	4.35	5.42	4.89
EBL (10^{-8} mM)	0.805	0.767	0.786	0.978	0.895	0.937	7.20	8.24	7.72	4.00	5.08	4.54
B (20 mg kg^{-1})	0.725	0.667	0.696	0.766	0.707	0.737	7.64	9.00	8.32	6.08	7.29	6.68
B (60 mg kg^{-1})	0.581	0.469	0.525	0.682	0.564	0.623	8.84	10.52	9.68	6.75	8.26	7.51
B (20 mg kg^{-1})+HBL	0.730	0.682	0.706	0.865	0.803	0.834	7.62	8.95	8.28	4.99	5.98	5.49
B (60 mg kg^{-1})+HBL	0.580	0.471	0.525	0.826	0.775	0.800	8.83	10.49	9.66	5.83	6.80	6.31
B (20 mg kg^{-1})+EBL	0.756	0.682	0.719	0.891	0.803	0.847	7.58	8.87	8.22	4.75	5.82	5.29
B (60 mg kg^{-1})+EBL	0.580	0.477	0.528	0.835	0.786	0.810	8.73	10.38	9.55	5.26	6.37	5.82
Mean	0.704	0.636		0.841	0.771		7.86	9.21		5.31	6.42	
LSD at 5%	V = 0.003 (Sig.)			V = 0.003 (Sig.)			V = 0.03 (Sig.)			V = 0.02 (Sig.)		
	T = 0.006 (Sig.)			T = 0.008 (Sig.)			T = 0.06 (Sig.)			T = 0.04 (Sig.)		
	V × T = 0.009 (Sig.)			V × T = 0.009 (Sig.)			V × T = 0.09 (Sig.)			V × T = 0.06 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.5.8 Electrolyte leakage

The plants raised in the soil amended with B (20 or 60 mg kg^{-1}) showed marked increase in the leaf electrolyte leakage at both the stages of growth (Table 55). The higher concentration of B triggered an increase in the leakage of the ions in both the varieties by 23% and 28% at 45 DAS and 16% and 22% at 60 DAS, in Varuna and Chapka Rohini, respectively, compared to their control plants. However, the foliar spray of BRs (10^{-8} M HBL/EBL) significantly checked the leakage of electrolytes under stress free condition compared to their water-sprayed control plants, at 60 DAS. EBL excelled in its effect compared to HBL and decreased the electrolyte leakage by 31% and 25% in Varuna and Chapka Rohini, respectively, compared to their control plants, at 60 DAS. Moreover, the application of HBL completely neutralized the effect of lowest concentration (20 mg kg^{-1}) of B and partially that of highest concentration (60 mg kg^{-1}), at 60 DAS. Varuna was more responsive than Chapka Rohini.

4.5.9 Carbonic anhydrase (CA) activity

The plants exposed to B (20 or 60 mg kg^{-1}) stress possessed significantly lower activity of CA in both the varieties (Varuna and Chapka Rohini), than their respective control plants (Table 56). However, the application of HBL or EBL to the foliage of stress-free plants significantly increased the activity of CA by 26% and 39% in Varuna and by 17% and 29% in Chapka Rohini, respectively, compared to their corresponding control plants, at 60 DAS. Furthermore, BRs analogues (HBL or EBL) completely nullified the damaging effect of both the concentrations (20 or 60 mg kg^{-1}) of B in Varuna and Chapka Rohini, at 60 day stage of growth.

4.5.10 Nitrate reductase (NR) activity

With the advancement of plant age from 45 to 60 DAS, the activity of NR increased in both the varieties, Varuna and Chapka Rohini (Table 56). Moreover, the application of either of the BRs further increased its activity in both the varieties, at both the stages of growth, compared to their control plants. In terms of percentage, the HBL increased the NR activity by 30% and 23% and EBL by 43% and 36% in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 60 day stage of growth. However, the activity was less in the plants raised with the application of B into the soil, at both the stages of growth. This damage was more pronounced in Chapka Rohini than Varuna but was overcome completely by the

Table 56: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-9} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and nitrate reductase activity (n mole NO_2 g^{-1} FM s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Carbonic anhydrase activity						Nitrate reductase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.31	1.81	2.06	2.44	2.11	2.28	450	407	429	581	540	561
HBL (10^{-8} mM)	2.75	2.06	2.40	3.07	2.47	2.77	551	466	508	753	668	711
EBL (10^{-8} mM)	2.91	2.15	2.53	3.39	2.73	3.06	585	488	536	833	733	783
B (20 mg kg^{-1})	2.16	1.64	1.90	2.38	2.00	2.19	409	357	383	549	489	519
B (60 mg kg^{-1})	1.79	1.27	1.53	1.99	1.54	1.77	325	262	293	455	368	412
B (20 mg kg^{-1})+HBL	2.43	1.98	2.21	2.83	2.34	2.58	468	449	458	709	622	665
B (60 mg kg^{-1})+HBL	1.82	1.36	1.59	2.66	2.22	2.44	334	274	304	640	580	610
B (20 mg kg^{-1})+EBL	2.50	1.98	2.24	2.97	2.46	2.71	473	445	459	733	655	694
B (60 mg kg^{-1})+EBL	1.91	1.42	1.66	2.73	2.28	2.51	348	282	315	677	609	643
Mean	2.29	1.74		2.72	2.24		438	381		659	585	
LSD at 5%	V = 0.06 (Sig.)			V = 0.05 (Sig.)			V = 11.7 (Sig.)			V = 17.39 (Sig.)		
	T = 0.19 (Sig.)			T = 0.28 (Sig.)			T = 24.80 (Sig.)			T = 29.40 (Sig.)		
	V × T = 0.28 (Sig.)			V × T = 0.36 (Sig.)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

exogenous application of HBL or EBL as a follow up treatment to the foliage, at 60 day stage of growth. The response of Varuna was better compared to Chapka Rohini.

4.5.11 Activity of antioxidant enzymes

The data depicted in tables 57 and 58 clearly revealed a significant increase in the activity of antioxidant enzymes (CAT, POX and SOD) irrespective of the treatment in both the varieties (Varuna and Chapka Rohini). Control plants possessed least activity of these enzymes. The level of all the three enzymes increased with an increase in the soil B content (20 or 60 mg kg^{-1}) and its level had an additive effect on the activity of these enzymes. Moreover, the follow up application of either of the analogues of brassinosteroid (10^{-8} M HBL/EBL) to the stressed plants further accelerated their activity in both the varieties. In terms of percentage, CAT, POX and SOD increased maximum by 66%, 79% and 84% in Varuna and 39%, 55% and 62% in Chapka Rohini plants that received B (60 mg kg^{-1}) plus EBL, respectively, compared to their control plants, at 45 day stage of growth. The variety Chapka Rohini possessed lesser antioxidant enzymes activity than Varuna.

4.5.12 Proline content

Leaf proline content in both the varieties (Varuna and Chapka Rohini) increased with the advancement of age from 45 to 60 DAS (Table 58) and the values improved further by treating the plants with B and/or BRs. Moreover, the leaf proline content increased by both B and BRs treatments. Of the two varieties, Varuna possessed higher proline content than Chapka Rohini. Moreover, the combination of EBL (10^{-8} M) and 60 mg kg^{-1} of B generated maximum values of proline which was 73% and 59% more at 45 DAS and 63% and 49% higher at 60 DAS, in Varuna and Chapka Rohini, respectively, as compared to their control plants.

4.5.13 Yield characteristics

The stress-free plants exposed to HBL or EBL (10^{-8} M) had significantly higher number of pods and seed yield per plant, over the control plants, at harvest (Tables 59 and 60). However, the maximum number of pod and the seed yield per plant were noted in the plants which received EBL spray under non-stressed conditions and the increase was 41% and 35% in Varuna and 36% and 32% in Chapka Rohini respectively, over their control plants. However, the plants raised in the soil amended with B (20 or 60 mg kg^{-1}) had significant reduction in yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass or seed yield per plant) in the

Table 57 Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-6} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) catalase (mM H_2O_2 decomposed g^{-1} FM) and peroxidase (units g^{-1} FM) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Catalase activity						Peroxidase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	ChapkaRohini	Mean	Varuna	Chapka Rohini	Mean
Control	415	340	378	482	388	435	12.67	9.08	10.88	16.86	11.97	14.42
HBL (10^{-8} mM)	494	387	440	537	430	484	16.47	10.99	13.73	20.74	14.00	17.37
EBL (10^{-6} mM)	527	401	464	578	449	514	17.61	11.99	14.80	22.09	14.84	18.46
B (20 mg kg^{-1})	464	370	417	526	415	470	14.70	10.26	12.48	18.71	12.93	15.82
B (60 mg kg^{-1})	565	437	501	616	463	540	18.12	11.71	14.92	23.27	14.00	18.64
B (20 mg kg^{-1})+HBL	510	393	452	566	442	504	17.23	11.53	14.38	21.41	14.60	18.01
B (60 mg kg^{-1})+HBL	617	455	536	689	503	596	19.51	12.80	16.16	24.45	15.68	20.06
B (20 mg kg^{-1})+EBL	530	423	476	593	458	525	18.24	12.35	15.30	22.93	15.56	19.25
B (60 mg kg^{-1})+EBL	692	475	584	718	509	613	22.68	14.07	18.38	26.47	17.24	21.85
Mean	532	406		589	451		17.47	11.64		21.88	14.54	
LSD at 5%	V = 11.78 (Sig.)			V = 12.52 (Sig.)			V = 0.35 (Sig.)			V = 0.44 (Sig.)		
	T = 15.00 (Sig.)			T = 17.39 (Sig.)			T = 0.75 (Sig.)			T = 0.93 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.06 (Sig.)			V × T = 1.31 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 58: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on and superoxide dismutase (units g^{-1} FM) activity and proline content (µmol g^{-1} FM) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Superoxide dismutase activity						Proline content					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	123	117	120	155	130	143	15.56	11.32	13.44	18.42	14.92	16.67
HBL (10^{-8} mM)	155	135	145	178	144	161	19.40	13.48	16.44	21.88	16.83	19.35
EBL (10^{-8} mM)	162	150	156	196	155	175	21.35	14.54	17.95	22.61	17.84	20.22
B (20 mg kg^{-1})	141	131	136	174	143	159	18.12	12.61	15.37	20.05	15.96	18.01
B (60 mg kg^{-1})	184	162	173	217	168	192	25.09	16.93	21.01	26.24	20.92	23.58
B (20 mg kg^{-1})+HBL	162	147	154	197	156	176	20.28	13.72	17.00	22.48	17.42	19.95
B (60 mg kg^{-1})+HBL	195	170	183	226	177	202	26.05	17.70	21.87	28.32	21.30	24.81
B (20 mg kg^{-1})+EBL	176	159	168	211	168	190	22.00	14.32	18.16	24.03	17.87	20.95
B (60 mg kg^{-1})+EBL	226	190	208	243	191	217	26.93	18.00	22.47	30.13	22.33	26.23
Mean	169	151		200	159		21.64	14.73		23.79	18.38	
LSD at 5%	V = 4.76 (Sig.)			V = 4.13 (Sig.)			V = 0.42 (Sig.)			V = 0.48 (Sig.)		
	T = 10.10 (Sig.)			T = 8.77 (Sig.)			T = 0.88 (Sig.)			T = 1.02 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.25 (Sig.)			V × T = 1.45 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 59: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Number of pods per plant			Number of seeds per pod		
	45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	224	195	210	12.43	11.43	11.93
HBL (10^{-8} mM)	295	245	270	12.51	11.46	11.99
EBL (10^{-8} mM)	317	269	293	12.57	11.50	12.04
B (20 mg kg^{-1})	206	172	189	11.40	10.16	10.78
B (60 mg kg^{-1})	172	137	155	10.00	9.02	9.51
B (20 mg kg^{-1})+HBL	280	232	256	11.74	10.26	11.00
B (60 mg kg^{-1})+HBL	246	208	227	10.96	9.32	10.14
B (20 mg kg^{-1})+EBL	290	240	265	12.30	10.89	11.60
B (60 mg kg^{-1})+EBL	254	212	233	11.37	9.91	10.64
Mean	254	213		11.70	10.44	
LSD at 5%	V = 12.57 (Sig.)			V = 0.41 (Sig.)		
	T = 18.69 (Sig.)			T = 0.87 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

two varieties (Varuna and Chapka Rohini) in a concentration dependent manner. The toxic effect of B was more pronounced in variety Chapka Rohini than in Varuna. Furthermore, brassinosteroid analogues (HBL or EBL) used as a follow up treatment to stressed plants completely overcome the adverse impact of B, in case of number of pods and seed yield per plant, in both the varieties, at harvest.

4.6 EXPERIMENT 6

This experiment was carried out to assess the ameliorative role of best suited analogue (EBL) of brassinosteroids in two contrasting varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss under both NaCl and B stress. Selection of NaCl and B treatments [NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}), NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}), and NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1})] was made on the basis of the observations in experiments 2, 3 and 4. The aforesaid NaCl and B treatment was given in the soil prior to sowing and the foliage of 44 days old plants was subsequently sprayed either with DDW (control) or EBL (10^{-8} M). All the agronomic and cultural practices were kept same as described in Experiment 1. Selected number of plants was sampled at 45 and 60 DAS to assess all the growth, photosynthetic and biochemical parameters (Experiment 1). The remaining plants were allowed to grow up to maturity and were harvested at approximately 120 DAS, to study the yield attributes.

4.6.1 Shoot and root length

The length of the shoot and root increased with the progression of growth from 45 to 60 DAS (Table 61). However, the plants raised in the soil amended with NaCl and B had significantly lesser values for shoot and root length in both the varieties (Varuna and Chapka Rohini) at the two stages (45 and 60 DAS) of growth. Out of the three combinations tested, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) triggered maximum damage and decreased the values of shoot length by 38% and 46%; root length by 44% and 53% in Varuna and Chapka Rohini, respectively, compared with their controls, at 60 DAS. The decrease was more prominent in Chapka Rohini than in Varuna. However, spray of EBL (10^{-8} M) to the foliage of stress-free plants significantly increased the shoot and root length by 69% and 50% in Varuna and 59% and 41% in Chapka Rohini, respectively, than their control plants, at 60 days stage of growth. Moreover, the deleterious effects generated on the shoot length by NaCl (2.8 or 4.2 dsm^{-1}) in combination with B (60 mg kg^{-1}) were completely neutralized by the follow

Table 60: Effect of foliar spray of 28-homobrassinolide (HBL; 10^{-8} mM) or 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied boron (B; 20 or 60 mg kg^{-1} soil) on 100 seed mass and seed yield per plant in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	100 seed mass			Seed yield per plant		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	311	283	297	7.64	6.11	6.88
HBL (10^{-8} mM)	315	285	300	9.72	7.47	8.59
EBL (10^{-8} mM)	319	287	303	10.31	8.04	9.18
B (20 mg kg^{-1})	292	246	269	7.26	5.51	6.39
B (60 mg kg^{-1})	261	212	236	6.01	4.13	5.07
B (20 mg kg^{-1})+HBL	301	266	284	9.21	7.00	8.11
B (60 mg kg^{-1})+HBL	288	254	271	8.16	6.34	7.25
B (20 mg kg^{-1})+EBL	304	269	287	9.56	7.20	8.38
B (60 mg kg^{-1})+EBL	289	255	272	8.47	6.54	7.50
Mean	298	262		8.47	6.48	
LSD at 5%	V = 7.45 (Sig.)			V = 0.25 (Sig.)		
	T = 11.21 (Sig.)			T = 0.52 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig.= Significant; NS = Non-significant

Table 61: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 60 mg kg^{-1} soil) on shoot and root length (cm) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot length						Root length					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	38.91	29.55	34.23	71.80	64.88	68.04	17.78	10.66	14.22	26.78	15.48	21.13
EBL (10^{-8} M)	39.47	29.75	34.61	121.39	103.32	112.36	18.00	10.78	14.39	40.18	21.88	31.03
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	28.05	18.33	23.19	54.65	45.16	49.90	12.41	6.39	9.40	20.01	9.98	14.99
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	26.49	17.27	21.88	51.09	41.22	46.16	11.13	5.50	8.32	18.13	8.67	13.40
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	22.25	14.56	18.40	44.63	35.07	39.85	9.33	4.58	6.96	15.00	7.20	11.10
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	28.86	18.60	23.73	84.78	72.05	78.42	12.76	6.46	9.61	30.19	16.72	23.45
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	26.75	17.43	22.09	80.98	68.56	74.77	12.61	5.59	9.10	29.09	14.22	21.65
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	22.18	14.79	18.49	65.40	56.85	61.12	9.47	4.39	6.93	18.82	9.12	13.97
Mean	29.12	20.04		71.84	60.89		12.94	6.79		24.77	12.91	
LSD at 5%	V = 0.91 (Sig.)			V = 2.39 (Sig.)			V = 0.50 (Sig.)			V = 0.99 (Sig.)		
	T = 1.81 (Sig.)			T = 3.60 (Sig.)			T = 1.01 (Sig.)			T = 1.98 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.43 (Sig.)			V × T = 2.80 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

up treatment of EBL (10^{-8} M). However, in case of root, EBL could overcome the damages of NaCl (2.8 dsm^{-1}) + B (60 mg kg^{-1}), at 60 day stage of growth.

4.6.2 Shoot and root fresh mass

The foliar application of EBL (10^{-8} M) to the stressed-free plants increased the shoot and root fresh mass as compared to control (stressed free and non-sprayed) plants in both the varieties, at 60 day stage of growth (Table 62). However, the plants raised in the soil supplemented with NaCl and B exhibited a significant reduction in fresh mass of shoot and root at 45 and 60 DAS. The presence of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) in soil caused maximum reduction of 40% and 46% in shoot and 44% and 52% in root of Varuna and Chapka Rohini, respectively, compared to their control plants, at 60 day stage of growth. However, the application of EBL (10^{-8} M) as a follow up treatment to the foliage of stressed plants completely neutralized the deleterious effects of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) and to a limited extent that of NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}), at 60 day stage of growth. Moreover, treatment of EBL was more effective in Varuna than Chapka Rohini.

4.6.3 Shoot and root dry mass

Shoot and root dry mass followed a pattern similar to that of the fresh mass (4.6.2). The exogenous application of EBL (10^{-8} M) increased the shoot and root dry mass in both Varuna and Chapka Rohini, at 60 DAS (Table 63). The increase was more pronounced in Varuna than Chapka Rohini, in terms of percentage, the values were higher by 82% and 77% in Varuna and 76% and 66% in Chapka Rohini, as compared to their respective control plants, at 60 day stage of growth. However, NaCl and B given together significantly decreased the shoot and dry mass of both varieties (Varuna and Chapka Rohini), at both the stages (45 and 60 DAS) of growth. Of the three stress treatments of NaCl and B tested, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) induced maximum toxicity and decreased the shoot dry mass by 41% and 49% and root dry mass by 46% and 52% in Varuna and Chapka Rohini, respectively, than their controls, at 45 DAS. The decrease was more prominent at an early stage (45) than at later stage (60) of growth. Moreover, the plants raised in the soil amended with NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) or NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) and sprayed with EBL could restore the values, which were comparable with that of control (stress free) plants, at 60 DAS.

Table 62: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root fresh mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	9.69	5.78	7.74	12.09	8.18	10.14	3.16	2.64	2.90	6.30	4.65	5.48
EBL (10^{-8} M)	9.78	5.91	7.85	19.58	12.27	15.93	3.22	2.67	2.95	10.47	7.39	8.93
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	7.27	3.64	5.46	9.54	5.72	7.63	2.06	1.43	1.75	4.58	2.9.6	3.77
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	6.64	3.33	4.98	8.20	5.00	6.60	1.84	1.29	1.56	4.04	2.52	3.28
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	5.48	2.89	4.19	7.07	4.39	5.73	1.65	1.10	1.37	3.54	2.24	2.89
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	7.38	3.69	5.53	13.90	8.91	11.40	2.13	1.48	1.80	6.92	4.79	5.85
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	6.78	3.37	5.08	13.30	8.26	10.78	1.89	1.35	1.62	6.61	3.88	5.24
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	5.21	2.84	4.02	10.73	6.77	8.75	1.60	1.07	1.34	5.36	3.49	4.43
Mean	7.28	3.93		11.80	7.44		2.19	1.63		5.98	3.99	
LSD at 5%	V = 0.25 (Sig.)			V = 0.42 (Sig.)			V = 0.09 (Sig.)			V = 0.27 (Sig.)		
	T = 0.50 (Sig.)			T = 0.84 (Sig.)			T = 0.18 (Sig.)			T = 0.38 (Sig.)		
	V × T = 0.70 (Sig.)			V × T = 1.19 (Sig.)			V × T = NS			V × T = 0.76 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 63: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on shoot and root dry mass (g) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Shoot dry mass						Root dry mass					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.79	1.80	2.30	3.58	2.40	2.99	0.90	0.74	0.82	1.51	1.07	1.29
EBL (10^{-8} M)	2.91	1.84	2.38	6.51	4.23	5.37	0.91	0.75	0.83	2.67	1.78	2.23
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	1.97	1.11	1.54	2.95	1.84	2.39	0.59	0.43	0.51	1.09	0.70	0.89
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	1.74	0.96	1.35	2.45	1.46	1.96	0.53	0.38	0.46	0.92	0.58	0.75
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	1.51	0.80	1.16	2.12	1.23	1.68	0.44	0.31	0.37	0.82	0.51	0.66
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	2.03	1.12	1.57	4.03	2.59	3.31	0.61	0.45	0.53	1.63	1.36	1.49
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	1.76	0.97	1.36	3.88	2.26	3.07	0.55	0.39	0.47	1.56	0.89	1.23
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	1.48	0.78	1.13	3.21	1.98	2.60	0.42	0.30	0.36	1.27	0.81	1.04
Mean	2.03	1.17		3.59	2.25		0.62	0.47		1.43	0.96	
LSD at 5%	V = 0.09 (Sig.)			V = 0.13 (Sig.)			V = 0.02 (Sig.)			V = 0.06 (Sig.)		
	T = 0.18 (Sig.)			T = 0.26 (Sig.)			T = 0.05 (Sig.)			T = 0.12 (Sig.)		
	V × T = 0.26 (Sig.)			V × T = 0.37 (Sig.)			V × T = 0.06 (Sig.)			V × T = 0.17(Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.6.4 Leaf area

Table 64 depicted that the leaf area increased with the advancement of plant age from 45 to 60 days. However, the presence of NaCl in combination with B in the soil significantly decreased the leaf area in the order of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) > NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) > NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}), in both the varieties (Varuna and Chapka Rohini), at both the stages (45 or 60 DAS). The decrease in leaf area was more prominent in Chapka Rohini than in Varuna. Moreover, the spray of EBL (10^{-8} M) to the foliage of stress free plants significantly increased the leaf area by 40% and 33% in Varuna and Chapka Rohini, respectively, compared to their control (stress free and non-sprayed) plants, at 60 DAS. Furthermore, the damage to the leaf area in the plants fed with NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) or (4.2 dSm^{-1}) + B (60 mg kg^{-1}) was completely overcome by the follow up treatment of EBL and partially with that of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) in Varuna. Besides this, in Chapka Rohini, EBL completely overcome the toxic effect of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) and partially that of other stress treatments, at 60 DAS. Varuna had higher values of leaf area than Chapka Rohini.

4.6.5 Chlorophyll content (SPAD value)

The leaf chlorophyll content (SPAD value) increased as the growth advanced from 45 to 60 DAS (Table 64). These values improved further by the foliar application of EBL (10^{-8} M) to stress free plants by 41% and 28% in Varuna and Chapka Rohini, respectively, over respective control plants, at 60 day stage of growth. However, the presence of NaCl + B in the soil had a negative impact, at both the stages of growth. The maximum loss in chlorophyll was observed in the presence of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) in the soil decreasing the values by 42% and 37% in Varuna and 50% and 46% in Chapka Rohini, compared to their control plants, at 45 and 60 DAS, respectively. However, the impact of NaCl (2.8 or 4.2 dSm^{-1}) + B (60 mg kg^{-1}) stress was completely overcome by the follow up treatment of EBL and partially that of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}), at 60 day stage of growth, more prominently in Varuna than Chapka Rohini.

4.6.6 Photosynthesis and related attributes

Net photosynthetic rate (P_N) and its related attributes viz. stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) increased substantially with the advancement of the plant age (Tables 65 and 66). The leaves of stress free and

Table 64: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on leaf area (cm^2) and chlorophyll content (SPAD value) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Leaf area						Chlorophyll content (SPAD value)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	35.17	32.32	33.75	43.65	36.32	39.99	42.14	29.29	35.72	56.74	35.10	45.92
EBL (10^{-8} M)	35.31	32.54	33.93	61.11	48.30	54.70	42.57	29.55	36.06	79.95	45.04	62.49
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	24.66	19.28	21.97	33.54	25.10	29.32	31.99	19.33	25.66	44.32	24.96	34.64
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	22.78	17.26	20.02	31.32	22.14	26.73	29.19	17.38	23.28	41.68	22.58	32.13
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	18.99	14.20	16.60	25.85	18.27	22.06	23.98	14.94	19.46	38.82	19.87	29.34
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	26.30	20.01	23.15	49.73	39.06	44.39	32.10	19.42	25.76	67.63	39.33	53.48
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	23.84	17.83	20.84	46.96	34.91	40.93	29.36	17.33	23.34	61.60	37.12	49.36
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	19.61	13.79	16.70	38.15	28.77	33.46	23.61	14.74	19.17	47.22	25.67	36.44
Mean	25.83	20.90		41.29	31.61		31.87	20.25		54.74	31.21	
LSD at 5%	V = 1.05 (Sig.)			V = 1.44 (Sig.)			V = 0.22 (Sig.)			V = 0.19 (Sig.)		
	T = 2.10 (Sig.)			T = 2.34 (Sig.)			T = 0.38 (Sig.)			T = 0.44 (Sig.)		
	V × T = NS			V × T = NS			V × T = 0.63 (Sig.)			V × T = 0.54 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 65: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Net photosynthetic rate						Stomatal conductance					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	20.42	15.20	17.81	30.11	18.05	24.08	0.067	0.051	0.059	0.086	0.069	0.078
EBL (10^{-8} M)	23.46	16.74	20.10	43.16	23.55	33.36	0.075	0.056	0.065	0.138	0.102	0.120
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	14.11	8.59	11.35	23.56	12.22	17.89	0.048	0.031	0.039	0.065	0.047	0.056
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	12.85	8.15	10.50	21.12	11.25	16.18	0.041	0.026	0.034	0.059	0.040	0.050
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	11.05	7.29	9.17	18.85	9.81	14.33	0.034	0.022	0.028	0.051	0.033	0.042
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	16.34	9.27	12.80	35.79	20.75	28.27	0.052	0.034	0.043	0.099	0.077	0.088
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	13.48	8.19	10.84	31.30	18.57	24.94	0.051	0.028	0.039	0.090	0.068	0.079
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	10.83	7.17	9.00	22.80	12.56	17.68	0.037	0.023	0.030	0.069	0.051	0.060
Mean	15.14	9.98		28.34	15.85		0.050	0.034		0.082	0.061	
LSD at 5%	V = 0.34 (Sig.)			V = 0.11 (Sig.)			V = 0.001 (Sig.)			V = 0.003 (Sig.)		
	T = 0.68 (Sig.)			T = 0.87 (Sig.)			T = 0.005 (Sig.)			T = 0.006 (Sig.)		
	V × T = 0.97 (Sig.)			V × T = 0.99 (Sig.)			V × T = 0.004 (Sig.)			V × T = 0.009 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 66: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O_2 m^{-2} s^{-1}) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Internal CO_2 concentration						Transpiration rate					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	327	253	290	379	282	331	3.49	2.48	2.99	4.63	3.41	4.02
EBL (10^{-8} M)	391	271	331	509	345	427	4.12	2.75	3.44	6.30	4.40	5.35
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	247	161	204	304	199	251	2.66	1.63	2.14	3.81	2.45	3.13
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	228	141	185	281	180	231	2.48	1.49	1.99	3.61	2.35	2.98
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	191	128	159	250	151	201	2.21	1.33	1.77	3.26	2.04	2.65
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	260	169	215	449	313	381	2.73	1.67	2.20	5.30	3.76	4.53
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	241	146	194	382	279	330	2.59	1.54	2.07	4.77	2.97	4.04
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	186	125	156	328	218	273	2.24	1.34	1.79	4.13	2.83	3.48
Mean	259	174		360	246		2.82	1.78		4.48	3.03	
LSD at 5%	V = 1.19 (Sig.)			V = 0.90 (Sig.)			V = 0.01 (Sig.)			V = 0.02 (Sig.)		
	T = 3.38 (Sig.)			T = 2.80 (Sig.)			T = 0.05 (Sig.)			T = 0.05 (Sig.)		
	V × T = 3.36 (Sig.)			V × T = 2.55 (Sig.)			V × T = 0.06 (Sig.)			V × T = 0.05 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

stressed plants, supplied with EBL (10^{-8} M) had higher photosynthetic attributes at both the stages of growth. Furthermore, application of EBL to stress free plants increased P_N by 43% and 30%; g_s by 60% and 48%; C_i by 34% and 22%; and E by 36% and 29% in Varuna and Chapka Rohini, respectively, over their control plants, at 60 DAS. However, the plants raised in the soil fed with NaCl + B exhibited a significant loss in the values of all the above photosynthetic parameters, at both the stages of growth (45 and 60 DAS) in both the varieties (Varuna and Chapka Rohini). The damage was more pronounced at 45 DAS compared to 60 DAS, in Chapka Rohini. Moreover, the follow-up application of EBL to the foliage of stressed plants completely nullified the effect of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) and partially that of NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) or NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}), at 60 day stage of growth. Varuna showed better photosynthetic response to the treatment than Chapka Rohini.

4.6.7 Maximum quantum yield of PSII

Table 67 revealed that the foliage of plants, that received EBL (10^{-8} M) had higher values for maximum quantum yield of PSII (Fv/Fm) at both the stages (45 and 60 DAS) of growth. At 60 day stage these values were 22% and 14% higher, compared to their control plants in Varuna and Chapka Rohini, respectively. However, the presence of NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and B (60 mg kg^{-1}) in the soil decreased these values, in a manner dependent on NaCl concentration. The higher concentration (5.6 dSm^{-1}) of NaCl in combination with B (60 mg kg^{-1}) triggered maximum toxicity and reduced Fv/Fm by 26% and 35% in Varuna and Chapka Rohini, respectively, compared to their control plants, at 60 day stage of growth. Moreover, the follow up treatment of EBL to the stressed plants completely overcome the deleterious effect of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) and partially that of the other treatments, at 60 day stage of growth.

4.6.8 Electrolyte leakage

The presence of salt and B in soil increased the leakage of ions from the leaves at both the stages (45 and 60 DAS) of growth more prominently at 45 DAS (Table 67). Out of the treatments tested, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) triggered maximum rate of leakage of electrolytes which was 34% and 41% more in Varuna and Chapka Rohini, respectively, as compared to their control plants, at 45 DAS. However, the application of EBL checked the loss of electrolytes in both the varieties, at 60 DAS.

Table 67: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on maximum quantum yield of PS II and electrolyte leakage (%) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Maximum quantum yield of PS II (Fv/Fm)						Electrolyte leakage (%)					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	0.769	0.742	0.756	0.800	0.763	0.782	7.49	8.36	7.93	5.94	6.50	6.22
EBL (10^{-8} M)	0.806	0.764	0.785	0.980	0.873	0.927	7.00	8.06	7.53	4.21	5.05	4.63
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	0.592	0.495	0.543	0.678	0.564	0.621	9.44	10.98	10.21	7.08	8.00	7.54
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	0.552	0.446	0.499	0.631	0.531	0.581	9.74	11.56	10.65	7.44	8.46	7.95
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	0.522	0.422	0.472	0.589	0.493	0.541	10.35	12.25	11.30	7.79	9.13	8.46
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	0.630	0.525	0.578	0.873	0.794	0.833	9.35	10.82	10.08	5.03	5.78	5.41
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	0.538	0.469	0.759	0.828	0.702	0.785	9.53	11.08	10.31	5.97	6.53	6.25
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	0.518	0.419	0.469	0.713	0.616	0.664	10.34	12.38	11.36	6.30	7.21	6.75
Mean	0.616	0.535		0.761	0.667		9.16	10.69		6.22	7.08	
LSD at 5%	V = 0.002 (Sig.)			V = 0.004 (Sig.)			V = 0.04 (Sig.)			V = 0.02 (Sig.)		
	T = 0.005 (Sig.)			T = 0.008 (Sig.)			T = 0.08 (Sig.)			T = 0.04 (Sig.)		
	V × T = 0.007 (Sig.)			V × T = 0.011 (Sig.)			V × T = 0.11 (Sig.)			V × T = 0.06 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Moreover, the spray of EBL as a follow-up treatment to the stressed plants completely overcome the toxic effects of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) whereas, the effect of NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) was also minimised and brought the values of electrolyte leakage to the level of the control, in both the varieties (Varuna and Chapka Rohini), at 60 day stage of growth.

4.6.9 Carbonic anhydrase (CA) activity

The activity of CA increased as the growth progressed from 45 to 60 DAS in both the varieties (Varuna and Chapka Rohini) of mustard (Table 68). However, the activity decreased significantly with the increase in the NaCl concentration (2.8, 4.2 or 5.6 dSm^{-1}) in combination with B (60 mg kg^{-1}). The treatment of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) in soil was most toxic and decreased the activity of CA by 37% and 32% in Varuna and 46% and 40% in Chapka Rohini, at 45 and 60 DAS, respectively, compared to their control plants. However, application of 10^{-8} M of EBL significantly increased the activity of CA in both the varieties (Varuna and Chapka Rohini), at the two stages (45 or 60 DAS) of growth. The maximum increase by EBL was noted at 60 DAS that was 39% and 31% more in Varuna and Chapka Rohini, respectively, compared to their control plants. Moreover, the impact generated by NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) or NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) was completely neutralized by the follow up treatment of EBL and partially that of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}), at 60 DAS.

4.6.10 Nitrate reductase (NR) activity

With the advancement of growth from 45 to 60 DAS, the activity of NR increased in both the varieties (Table 68). The application of EBL (10^{-8} M) to the foliage of non-stressed (control) plants further increased the activity of NR at both the stages of growth. However, the maximum increase was noted at 60 DAS which was 43% and 35% more in Varuna and Chapka Rohini, respectively, in comparison to their control plants. However, the plants grown in the soil with different concentrations (2.8, 4.2 or 5.6 dSm^{-1}) of NaCl in combination with B (60 mg kg^{-1}) significantly reduced the activity of NR and the degree of damage was in proportion to the concentrations of NaCl. However, complete recovery against NaCl (2.8 or 4.2 dSm^{-1}) and B (60 mg kg^{-1}) stress was found in 60 day old plants that were subsequently applied with EBL. Amongst the two varieties, Varuna was more tolerant to stress than Chapka Rohini.

Table 68: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and nitrate reductase (n mole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Carbonic anhydrase activity						Nitrate reductase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	2.26	1.73	2.00	2.47	2.06	2.27	429	328	379	562	430	496
EBL (10^{-8} M)	2.81	2.07	2.44	3.44	2.72	3.08	558	390	474	803	580	692
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	1.83	1.23	1.53	1.94	1.51	1.73	317	215	266	436	300	368
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	1.58	1.07	1.32	1.88	1.42	1.65	292	196	244	398	276	337
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	1.42	0.93	1.17	1.67	1.23	1.45	251	166	209	359	244	301
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	2.01	1.32	1.66	2.89	2.31	2.60	347	231	289	662	487	574
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	1.65	1.13	1.39	2.67	2.20	2.44	309	183	246	610	454	532
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	1.46	0.96	1.21	2.05	1.43	1.74	260	173	216	446	344	376
Mean	1.88	1.31		2.38	1.86		345	235		535	384	
LSD at 5%	V = 0.04 (Sig.)			V = 0.05 (Sig.)			V = 7.43 (Sig.)			V = 11.26 (Sig.)		
	T = 0.12 (Sig.)			T = 0.16 (Sig.)			T = 14.86 (Sig.)			T = 22.51 (Sig.)		
	V × T = 0.11 (Sig.)			V × T = 0.14 (Sig.)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

4.6.11 Activity of antioxidant enzymes

The data presented in tables 69 and 70 clearly revealed that the activity of antioxidant enzymes (CAT, POX and SOD) increased with the advancement of age from 45 to 60 DAS. Control plants (stress free, not supplied with EBL) possessed minimum activity of the antioxidant enzymes (CAT, POX and SOD). The activity of all these enzymes increased in the plants exposed to NaCl and B stress, the values improved further with the application of hormone (EBL; 10^{-8} M). The maximum activity of these enzymes was noted in plants administered with NaCl (5.6 dsm^{-1}) + B (60 mg kg^{-1}) and also received foliar treatment of EBL in both the varieties. In terms of percentage, the activity of CAT enzyme increased by 83% and 69%; POX by 98% and 89%; and SOD by 112% and 98% in Varuna and Chapka Rohini, respectively, than their control plants, at 45 DAS. Chapka Rohini possessed lesser antioxidant enzyme activity than Varuna.

4.6.12 Proline content

The plants raised in the soil amended with different concentrations (2.8, 4.2 or 5.6 dSm^{-1}) of NaCl in combination with B (60 mg kg^{-1}) had higher proline content which was in proportion to the concentrations of NaCl (Table 70). Furthermore, EBL (10^{-8} M) application to the stressed plants had an additive effect. Moreover, the plants exposed to NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) and also applied with EBL as a follow-up treatment, possessed highest proline content at both the growth stages. The aforesaid combination increased the proline accumulation by 120% and 96% in Varuna and Chapka Rohini respectively, as compared to their control plants, at 45 DAS. Moreover, the values were higher at 60 day stage than at 45 day stage of growth. Out of the two varieties, Varuna possessed high proline content than Chapka Rohini, at both the stages of growth.

4.6.13 Yield characteristics

The plants raised in the soil supplemented with NaCl and B had poor yield characteristics (number of pods per plant, number of seeds per pod, seed yield per plant and 100 seed mass) in both the varieties, Varuna and Chapka Rohini, at harvest (Tables 71 and 72). However, the exogenous application of EBL (10^{-8} M) to the foliage of stress free plants significantly increased the number of pods per plant by 39% and 36% and seed yield per plant by 35% and 31% in Varuna and Chapka Rohini, respectively, compared to their control plants. Moreover, the foliar spray of EBL to the stressed plants completely neutralized the toxic effects of NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) in both the varieties (Varuna and Chapka Rohini) and also that of NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) in Varuna only, for number of pods and seed yield per plant.

Table 69: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on catalase (mM H_2O_2 decomposed g^{-1} FM) and peroxidase (units g^{-1} FM) activity in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Catalase activity						Peroxidase activity					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	406	321	364	448	352	400	11.95	8.78	10.37	16.06	11.17	13.62
EBL (10^{-8} M)	535	385	460	546	397	472	18.28	11.84	15.06	22.64	13.96	18.30
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	557	409	483	572	430	501	17.43	11.62	14.52	22.37	14.06	18.22
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	595	439	517	624	463	543	18.63	12.54	15.59	23.14	14.98	19.06
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	637	470	554	670	495	583	19.44	13.28	16.36	24.12	15.68	19.90
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	604	442	523	625	460	542	18.94	13.04	15.99	24.04	15.67	19.86
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	662	494	578	716	510	613	20.88	14.29	17.58	26.46	17.58	22.02
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	745	545	645	766	547	657	23.70	16.60	20.15	29.47	19.51	24.49
Mean	593	438		621	457		18.66	12.75		23.54	15.33	
LSD at 5%	V = 15.66 (Sig.)			V = 10.93 (Sig.)			V = 0.37 (Sig.)			V = 0.49 (Sig.)		
	T = 22.84 (Sig.)			T = 24.86 (Sig.)			T = 0.74 (Sig.)			T = 0.97 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.05 (Sig.)			V × T = 1.38 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 70: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on superoxide dismutase (units g^{-1} FM) activity and proline content (µmol g^{-1} FM) in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Superoxide dismutase activity						Proline content					
	45 DAS			60 DAS			45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	130	112	121	162	142	152	16.46	12.32	14.39	19.42	16.20	17.81
EBL (10^{-8} M)	177	137	157	203	169	186	24.36	15.40	19.88	23.69	19.73	21.71
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	208	161	185	237	190	214	27.24	18.90	23.07	29.97	23.11	26.54
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	220	176	198	249	201	225	28.89	20.50	24.70	32.89	24.57	28.73
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	245	191	218	288	237	263	31.13	21.37	26.25	34.96	26.37	30.66
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	232	181	207	259	209	234	29.55	19.47	24.51	32.43	25.09	28.76
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	248	196	222	287	226	256	31.54	21.74	26.64	34.86	27.49	31.18
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	276	222	249	311	254	283	36.15	24.26	30.21	38.54	29.61	34.08
Mean	217	172		249	204		28.16	19.24		30.85	24.02	
LSD at 5%	V = 4.52 (Sig.)			V = 5.46 (Sig.)			V = 0.58 (Sig.)			V = 0.64 (Sig.)		
	T = 9.04 (Sig.)			T = 10.91 (Sig.)			T = 1.16 (Sig.)			T = 1.27 (Sig.)		
	V × T = NS			V × T = NS			V × T = 1.65 (Sig.)			V × T = 1.80 (Sig.)		

V = Varieties; T = Treatments; Sig. = Significant; NS = Non-significant

Table 71: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on number of pods per plant and number of seeds per pod in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	Number of pods per plant			Number of seeds per pod		
	45 DAS			60 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	232	192	212	12.19	8.95	10.57
EBL (10^{-8} M)	322	261	292	12.48	9.01	10.74
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	178	132	155	9.69	6.53	8.11
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	162	118	140	9.02	5.93	7.48
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	142	97	120	8.12	5.20	6.66
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	263	199	231	10.99	7.48	9.24
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	244	160	202	10.09	6.67	8.38
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	188	143	166	8.91	5.94	7.42
Mean	216	163		10.19	6.96	
LSD at 5%	V = 7.27 (Sig.)			V = 0.35 (Sig.)		
	T = 14.53 (Sig.)			T = 0.70 (Sig.)		
	V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 72: Effect of foliar spray of 24-epibrassinolide (EBL; 10^{-8} mM) against soil applied NaCl (2.8, 4.2 or 5.6 dSm^{-1}) and boron (B; 20 or 60 mg kg^{-1} soil) on 100 seed mass and seed yield per plant in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss at 45 and 60 DAS.

	100 seed mass			Seed yield		
	120 DAS			120 DAS		
	Varuna	Chapka Rohini	Mean	Varuna	Chapka Rohini	Mean
Control	308	255	282	8.74	6.89	7.82
EBL (10^{-8} M)	316	258	287	11.80	9.07	10.43
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})	257	192	225	6.87	4.67	5.77
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})	233	177	205	6.37	4.41	5.39
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})	213	157	185	5.64	3.82	4.73
NaCl (2.8 dSm^{-1})+B (60 mg kg^{-1})+EBL	256	198	227	10.02	7.15	8.58
NaCl (4.2 dSm^{-1})+B (60 mg kg^{-1})+EBL	263	205	234	9.36	5.77	7.56
NaCl (5.6 dSm^{-1})+B (60 mg kg^{-1})+EBL	219	171	195	7.66	5.21	6.44
Mean	258	202		8.31	5.87	
LSD at 5%	V = 8.83 (Sig.)			V = 0.33 (Sig.)		
	T = 17.65 (Sig.)			T = 0.64 (Sig.)		
	V × T = NS			V × T = 0.95 (Sig.)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Chapter 5

DISCUSSION

DISCUSSION

Plants are exposed to a variety of abiotic stresses such as salinity, drought, chilling and heat, heavy metals and excess micro-nutrients. The response of the plants to these stresses in a unique and complex way depends upon their degree of plasticity involving various morphological, anatomical, cellular, and physiological changes (Tuteja, 2007). Among them, essential micro-nutrients (especially B) and soil desiccating factors (salinity) are important sub-terrenial checks on plant growth. In the absence of these essential micro-nutrients, plants cannot complete their life cycle but the level of these elements prove fatal above permissible limit and could lead to cellular injuries and in extreme cases death of plants resulting into the loss of crop production worldwide (Barker, 2006). Moreover, nutritional stress has increased at an accelerating pace in recent years due to various anthropogenic activities and non-judicious application of fertilizers by farmers.

Salinity is another major serious environmental constraint that poses a severe threat to the growth of plants and thereby productivity. Seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also poor quality of the crop produce (Cavusoglu *et al.,* 2007). Phytohormones are known to play vital roles in improving the ability of plants to tolerate various abiotic stresses, by mediating growth, development, source/sink transitions and nutrient allocation (Fahad *et al.,* 2015). Out of the established class of plant growth regulators (PGRs), brassinosteroids (BRs) are steroidal group of plant hormones which help to promote growth, delay senescence, increase flower and fruit set, repel pests and most importantly overcome various abiotic stresses (Bajguz and Hayat, 2009).

The enzyme, carbonic anhydrase (CA) is a ubiquitous Zn containing metalloenzyme which catalyses the inter-conversion of CO_2 and HCO_3^- in living organisms. CA also plays an essential role in facilitating the transport of CO_2 and protons in the intracellular space, across biological membranes and in the layers of the extracellular space which are involved in the respiration and photosynthesis. The activity of CA in plants is largely determined by photon flux density, concentration of CO_2, availability of Zn (Tiwari *et al.,* 2005) and/or genetic expression (Kim *et al.,* 1994). Moreover, CA ensures the constant supply of inorganic carbon to

ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), at the level of grana of the chloroplast in actively photosynthesizing cells (Price *et al.,* 1994; Henry, 1996). In the present study, the plants treated with varying levels (20, 30, 40, 50 or 60 mg kg^{-1}) of B or NaCl (2.8, 4.2 or 5.6 dSm^{-1}) alone had lower activity of CA (Tables 8, 20, 32 and 44). Decrease in CA activity here could be due to the ability of B to form borate esters by binding to the ribose moieties of molecules such as ATP, NADH or NADPH that inhibit protein synthesis (Reid *et al.,* 2004) and consequently metabolic disruption (Reid, 2007b; Herrera-Rodrigues *et al.,* 2010). The loss in CA activity, under B-stress has also been reported in *Vigna radiata* (Yusuf *et al.*, 2011) and in *Brassica juncea* (Varshney *et al.*, 2015). On the other hand, salinity causes the closure of stomata, thereby decreasing the partial pressure of CO_2 as well as the level of internal CO_2 concentration and consequently the activity of CA because its activity is largely regulated by CO_2 concentration (Chaves *et al.,* 2009). Moreover, the salinity induced negative impact on the gene expression of CA synthesis (Liu *et al.,* 2012) could be an additional reason for the observed decrease in its activity. A similar concept has also been proposed by Tavallali *et al.* (2009), Liu *et al.* (2012) and Fariduddin *et al.* (2012) under salt stress. Therefore, application of NaCl in combination with B in soil might have imparted greater loss in CA activity in our observation (Tables 20, 32 and 44). However, foliar application of BRs (HBL/EBL) alone to stress-free or as a follow up treatment to B or B plus NaCl stressed plants elevated the activity of CA (Tables 56 and 68) by enhancing the expression of genes that encode related enzymes of the Calvin cycle which play an important role in the regeneration of Rubisco, a key enzyme in photosynthetic carbon fixation and improving the rate of CO_2 assimilation (Tables 53 and 65; Yu *et al.,* 2004). Thus, CA improves the activity of Rubisco and maximizes the carboxylation rate of Rubisco (Xia *et al.,* 2009). BRs mediated increase in CA activity has been reported earlier in *Vigna radiata* under B stress (Yusuf *et al.,* 2011), in *Lycopersicon esculentum*, under Cd stress (Hayat *et al.,* 2010c) and in *Brassica juncea* and *Cucumis sativus*, under salt stress (Alyemeni *et al.,* 2013; Fariduddin *et al.,* 2013).

The other enzyme, nitrate reductase (NR) catalyzes NAD(P)H mediated reduction of nitrate (NO_3^-) to nitrite (NO_2^-) (Campbell, 1999), to ensure the adequate supply of nitrogen in the plants for proper growth and productivity (Srivastava, 1995). This process of nitrate reduction depends on three main factors (a) substrate (NO_3^-)

level in the cytoplasm (b) the level of functional NR and/or (c) the activity level of functional NR. Each of these processes is directly or indirectly dependent on the metabolic sensors and/or signal transducers (Campbell, 1999) and transporters (Loque *et al,* 2003). However, the major rate limiting step in the process of nitrate reduction catalyzed by NR is the conversion of NO_3^- to NO_2^- which declined significantly by the presence of B and/or NaCl in the soil (Tables 8, 20, 32 and 44). This might be an after effect of the inhibition and/or metabolic dysfunction of NR (Campbell, 1999; Hopkins and Huner, 2004). In addition to this, stress factors interfere with the activity of plasmamembrane bound proton pump (Obata *et al.,* 1996), the structure and fluidity of the plasma membrane (Alia-Mohanty and Saradhi, 1992; Karim *et al.,* 1999). This could have restricted the uptake of NO_3^-, the inducer and substrate of the NR resulting in the decreased NR activity (Campbell, 1999). The physiology of NO_3^- uptake is also sensitive to inhibitors of protein synthesis. On exposure of plants to external nitrate, the rate of uptake of NO_3^- increases from two- to five folds, but addition of inhibitors of protein synthesis causes the rate to fall rapidly back to the constitutive level (Hopkins and Huner, 2008). Therefore, a decrease in NR activity in present study could be due to the ability of B to interfere with transcription and/or translation mechanisms by binding to ribose leading to protein synthesis inhibition (Reid, 2010). Similarly, Mahboobi *et al.* (2002) reported the decrease in NR activity in the root and leaf tissues of barley and wheat cultivars under B stress due to drastic decrease in nitrate uptake and leaf NO_3^- content. On the other hand, reduced NR activity in salt-stressed plants is attributed due to lower activity of nitrate transporters, proton pump and altered fluidity of membrane at root hair zone, inhibiting uptake and transport of nitrate to the shoot (Peuke and Jeschke, 1999; Debouba *et al.,* 2007; Dalio *et al.,* 2013). The loss in NR activity under salt stress is also in conformity with the studies of Anuradha and Rao (2003), Shahid *et al.* (2011) and Hayat *et al.* (2014). Therefore, all these altered processes led to the greater loss in NR activity in mustard plants which were exposed to NaCl and B treatment simultaneously (Tables 20, 32 and 44). However, application of BRs to stress-free or as a follow-up treatment to B and/or NaCl stressed plants, improved the NR activity (Tables 56 and 68) that could be due to an expression of BRs, over that of NaCl/B, on translation and/or transcription machinery (Khripach *et al.,* 2003) of NR and on the integrity of cell membranes (Dalio *et al.,* 2013). BRs are also involved in the regulation of plasma

membrane anion channels and proton pumps (Zhang *et al.,* 2005) which may have facilitated the easy uptake of nitrate (NO_3^-), the substrate for NR and thus, enhanced its activity. Furthermore, the involvement of BRs in increasing the level of substrate (NO_3^-) by acting at the level of cell membrane as BRI1 peptide has basic residues at P-3, P-4, P-6 and a hydrophobic residue at P-5, related to phosphorylated Ser which is similar to regulatory phosphorylation sites of sucrose-phosphate synthase (SPS), nitrate reductase and HMGCoA reductase (HMR) in their sequence of amino acids (Man-Ho *et al.,* 2000).

The plants grown under B and/or NaCl stress had lower chlorophyll content (SPAD value) in both the varieties of mustard (Tables 4, 16, 28 and 40). Decrease in chlorophyll content under B stress attributed to the overproduction of reactive oxygen species (ROS) under B stress (Reid *et al.,* 2004; Camacho-Cristobal *et al.,* 2008; Han *et al.,* 2009) which damages the reaction centre of photosystems located in thylakoid membranes and resulting to photo-oxidative damages in chlorophyll molecules (Papadakis *et al.,* 2004). The other possible reason could also be due the increased membrane permeability (Tables 7, 19, 31 and 43; Siddiqui *et al.,* 2013) by disrupting the membranous structures including that of grana (Somashekaraiah *et al.,* 1992; Luna *et al.,* 1994). These studies are in conformity with other crops like, *Vigna radiata* (Hasnain *et al.,* 2011), *Phaseolus vulgaris* (Nagesh *et al.,* 2012) and *Raphanus sativus* (Siddiqui *et al.,* 2013). On the other hand, salt stress also challenges the uptake of essential nutrients including Mg and Fe (Jia *et al.,* 2008) whose scarcity adversely affects the cellular biosynthesis of chlorophyll. The deficiency of these nutrients plays an important role in chlorosis, as Fe^{2+} and Mg^{2+} serve as essential cofactors of the polypeptide enzymes in PSI and PSII. It also leads to the degradation of biomembranes as evident from the increased membrane lipid peroxidation (Katsuhara *et al.,* 2005; Koca *et al.,* 2007). Moreover, the increased membrane permeability with an accumulated aminolevulinic acid (ALA; an initial precursor of chlorophyll) could be another major factor leading to decreases in chlorophyll content at higher salt stress (Lee, 2006). Therefore, a cumulative additive effect of B and salt stress generated more drastic decrease in chlorophyll content (Tables 16, 28 and 40). Our results showed responses similar to those reported by Lee (2006) in hot pepper plants under B and salt stress. However, plants sprayed with BRs (HBL/EBL) favoured the chlorophyll content in unstressed (control), B or B plus NaCl-stressed conditions

(Tables 52 and 64). BRs induce the expression of specific genes responsible for the synthesis of enzymes responsible for chlorophyll biosynthesis and others involved in photosynthesis (Yu *et al.*, 2004; Bajguz and Asami, 2005). Therefore, the most likely reason for supporting the increase in chlorophyll content is that BRs might have directly or indirectly encouraged chlorophyll biosynthesis (Hayat *et al.*, 2011a). Brassinosteroids also possibly retard the rate of degradation of chlorophyll molecules and that of the proteins associated with them, in particular the proteins of light-harvesting complexes located in thylakoid membranes (Hola, 2011).

The present study revealed that B and NaCl alone or in combination induced a significant decline in net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) in both the varieties (Tables 5-7, 17-19, 29-31 and 41-43). The decrease in stomatal conductance accompanied by a decrease in net photosynthetic rate could due to stomatal factors under the excess of B (Sotiropoulos *et al.*, 2002). Moreover, B stress resulted in a reduction of P_N and g_s due to stomatal closure has also been found in summer squash (Lovatt and Bates, 1984), orange (Papadakis *et al.*, 2004) and citrus (Han *et al.*, 2009). Increased levels of B also reduced the level of internal CO_2 concentration which lead to the reduction in absorbed photon-energy captured by the light harvesting system utilized in the electron transport system further decreased the photosynthesis (Landi *et al.*, 2012). The other possible reason for the reduction of P_N, g_s, C_i and E under B stress is the induced structural damage of thylakoids which affects the photosynthetic transport of electrons, as indicated by the reduction of the ratio between variable fluorescence and initial fluorescence (F_v/F_0) (Pereira *et al.*, 2000). Similarly, various authors have reported that B toxicity induces a loss in photosynthesis in several plants (Han *et al.*, 2009; Soylemezoglu *et al.*, 2009; Sheng *et al.*, 2010; Guidi *et al.*, 2011; Wang *et al.*, 2011; Ruuhola *et al.*, 2011; Chen *et al.*, 2012). However, the decrease in photosynthetic rate and its related attributes under salt stress is naturally due to the closure of stomata and suppression in the stomatal conductance (Flexas *et al.*, 2004; Chaves *et al.*, 2009). Salinity brings about the closure of stomata due to salt-induced ABA accumulation (Yang and Lu, 2005), thereby decreasing the partial pressure of CO_2 in the stroma (Iyengar and Reddy, 1996) that becomes the main reason for the observed loss of g_s, C_i and E in the present study (Tables 17-19, 29-31 and 41-43). Furthermore, it has also been reported that the increase in salt level decreases the carboxylation activity of Rubisco, an important enzyme in CO_2 fixation (Sivakumar

et al., 2000; Kahrizi *et al.*, 2012). NaCl is also reported to damage the structure and/or integrity of chlorophyll molecules and the ultrastructure of chloroplast (Guan *et al.*, 2013; Xing *et al.*, 2013) by disturbing the uptake of several essential nutrients like K, Ca, Mg, Mn, and Fe (Jia *et al.*, 2008), which hampers the functioning of photosynthetic electron transport chain and thus net photosynthesis (Sudhir *et al.*, 2005). Moreover, stress-induced activation of the process of senescence and a shift in the activity of related enzymes as a result of the changes in cytoplasmic structure and negative feedback of reduced sink activity (Iyengar and Reddy, 1996) and slowed pace of transport of photosynthates, under potassium deficiency (Cakmark, 2005) also caused a significant reduction in the rate of photosynthesis. Thus, all these factors cumulatively affected net photosynthetic rate (P_N) and their related attributes (g_s, C_i and E) under NaCl and/or B stress. The decrease in carbonic anhydrase activity (Tables 8, 20, 32 and 44) and chlorophyll content (Tables 4, 16, 28 and 40) are the other reasons to justify further decrease of net photosynthetic rate under NaCl and/or B-stressed conditions. A positive correlation between carbonic anhydrase activity and net photosynthetic rate (Figures 1-6) as well as between chlorophyll content (SPAD level) and net photosynthetic rate (Figures 7-12) further corroborate the present observations. The support is also gained from the similar observations of other researchers (Noreen *et al.*, 2010; Akram and Ashraf, 2011; Ahmad *et al.*, 2012; Eisa *et al.*, 2012; Wu *et al.*, 2012).

The recovery in net photosynthetic rate (P_N) and its attributes (g_s, C_i and E) in the B and/or NaCl-stressed plants by exposing them to BRs (HBL or EBL) (Tables 53-54 and 65-66) may be attributed to the involvement of BRs in activating the two important enzymes that initiate the process of photosynthesis i.e., CA and Rubisco (Yu *et al.*, 2004). Moreover, BRs had a positive effect on the activation of Rubisco through an improvement in maximum carboxylation rate of Rubisco (Vc,max). Activation of Rubisco by BRs takes place through enhanced expression of genes encoding the enzymes of Calvin cycle which might play a positive role in RuBP regeneration/(Jmax), thereby increasing maximum carboxylation rate (Vc,max) of Rubisco (Xia *et al.*, 2009). In addition to this, the higher activity of CA increases the capacity of CO_2 assimilation in the Calvin cycle which is mainly attributed to efficient functioning of Rubisco (Bajguz and Asami, 2005). All these in a cumulative action speeded up the net photosynthetic rate (P_N) of the plants (Hola, 2011 and Tables 53 and 65). These observations are further corroborated by the computed positive

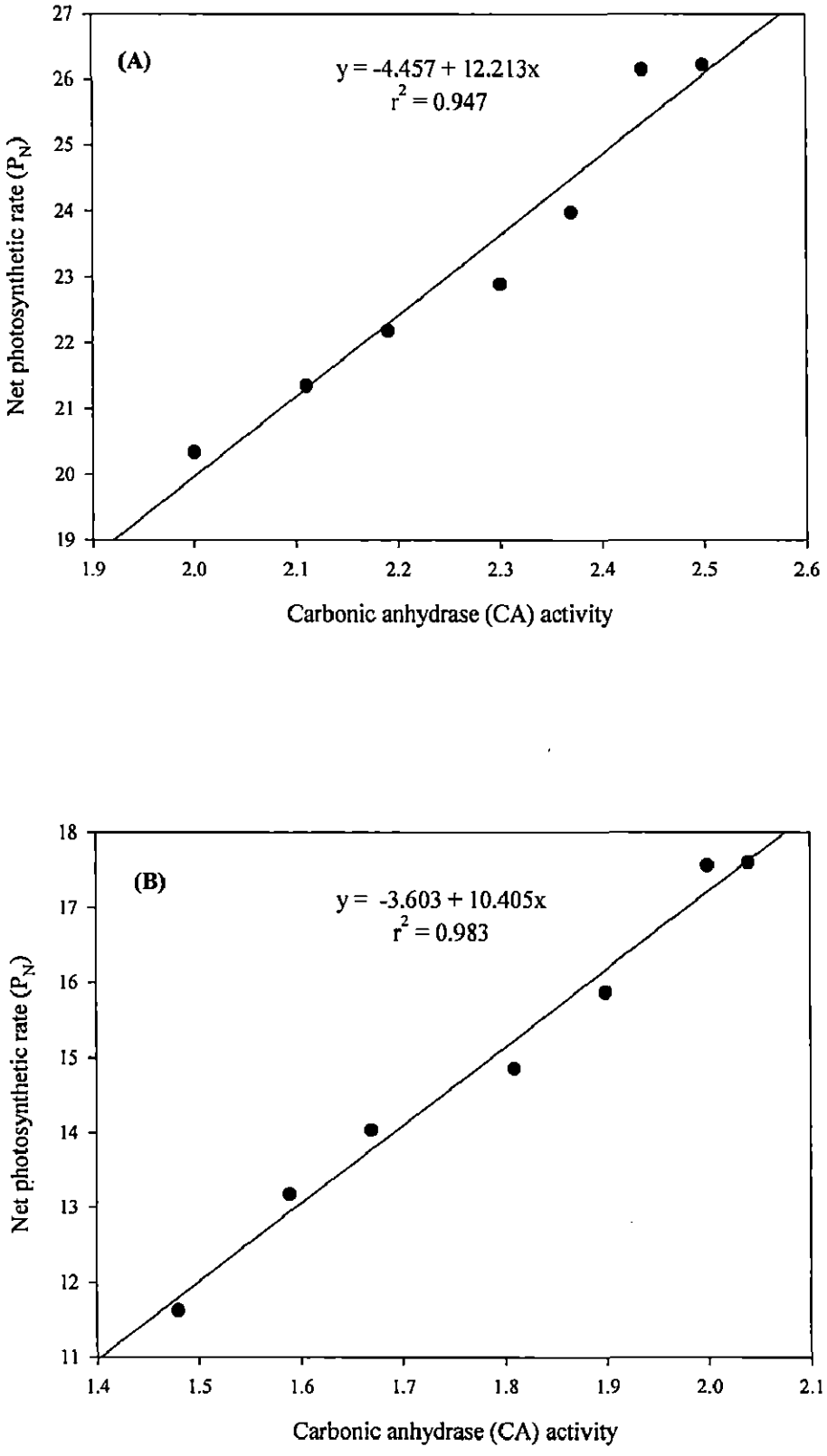
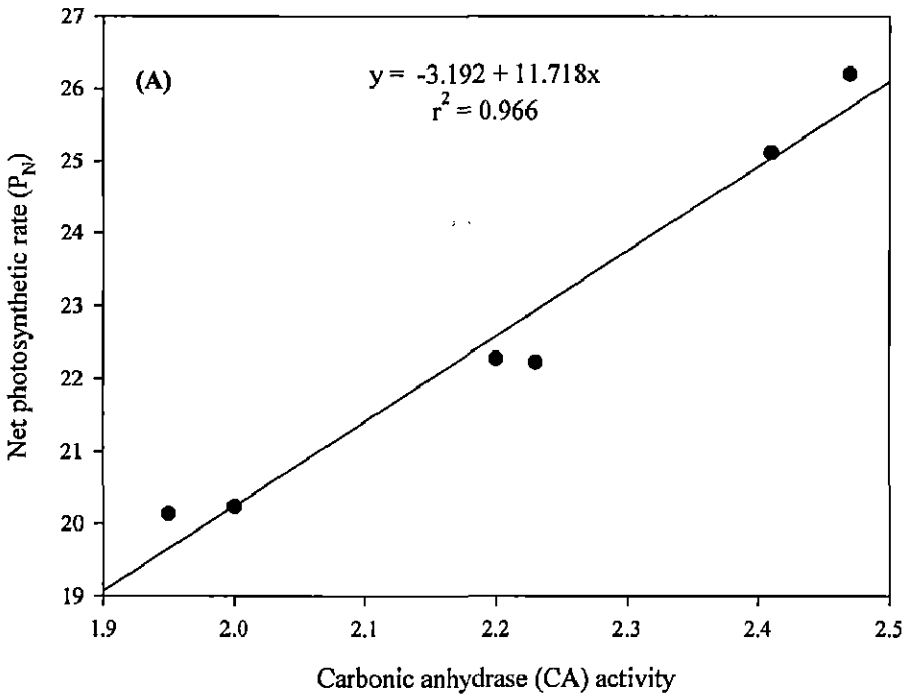


Figure 1: Correlation coefficient values between carbonic anhydrase (CA) activity and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 1)



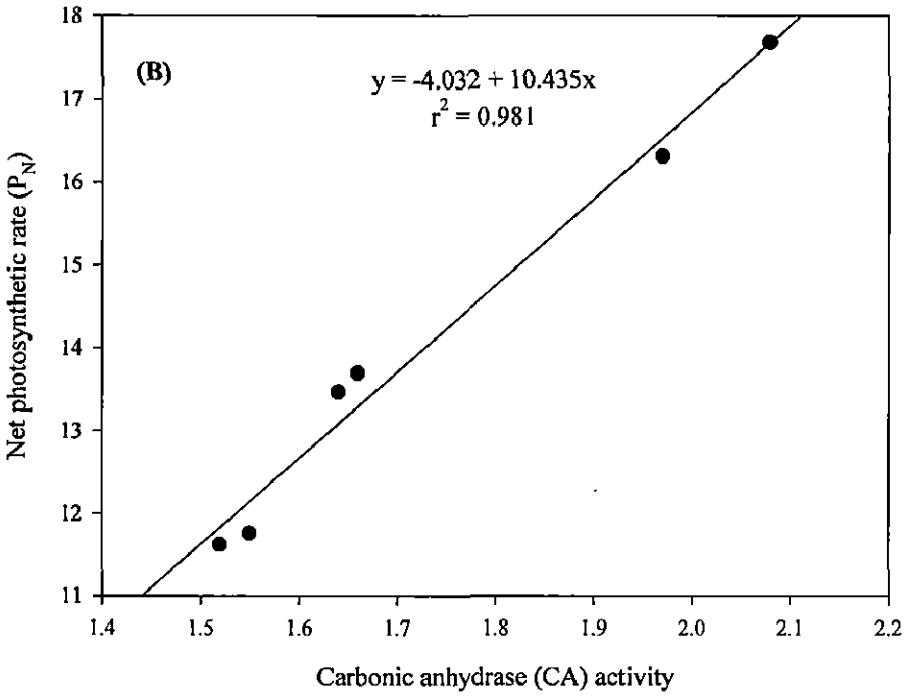


Figure 2: Correlation coefficient values between carbonic anhydrase (CA) activity and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 2)

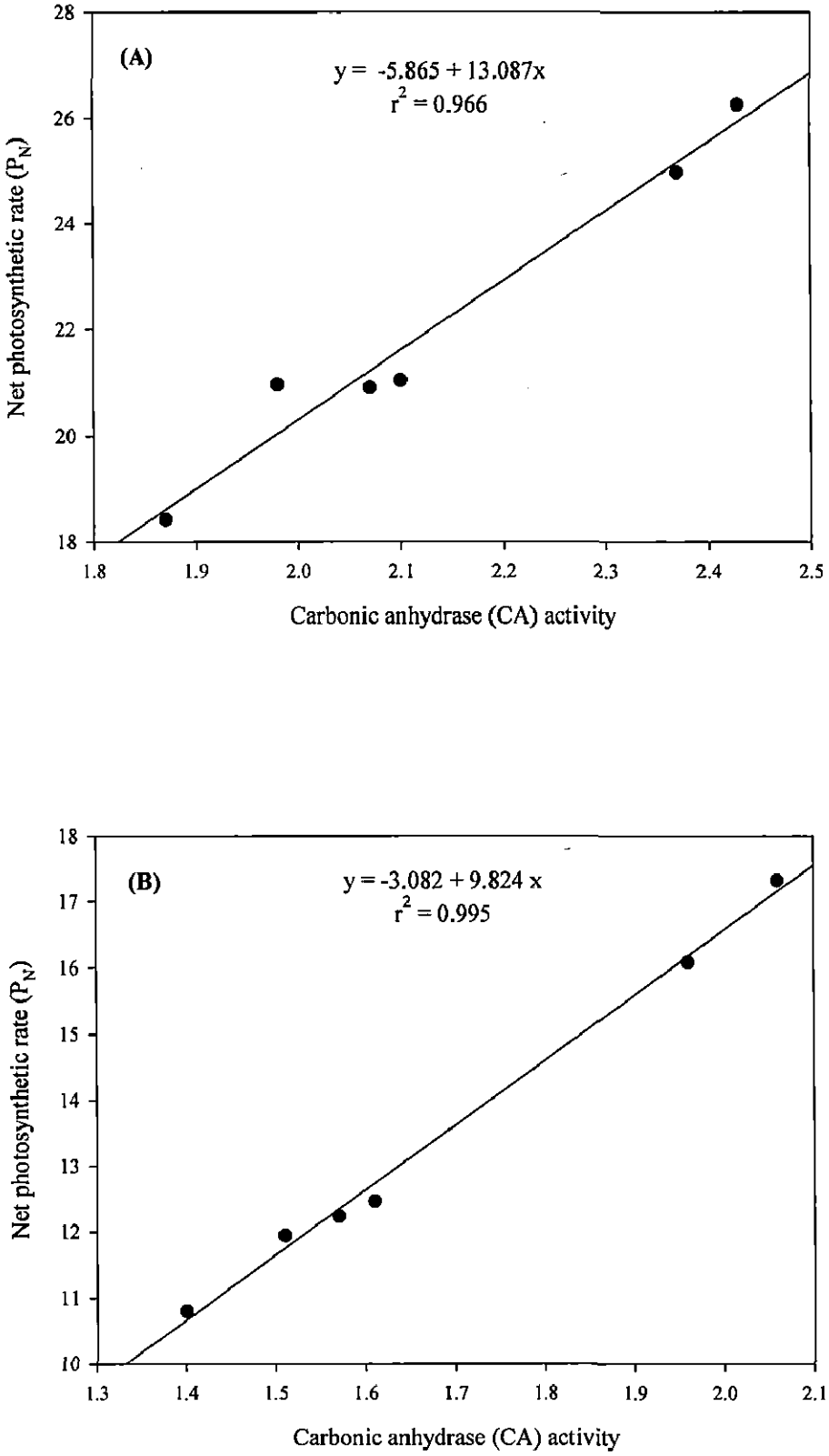


Figure 3: Correlation coefficient values between carbonic anhydrase (CA) activity and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 3)

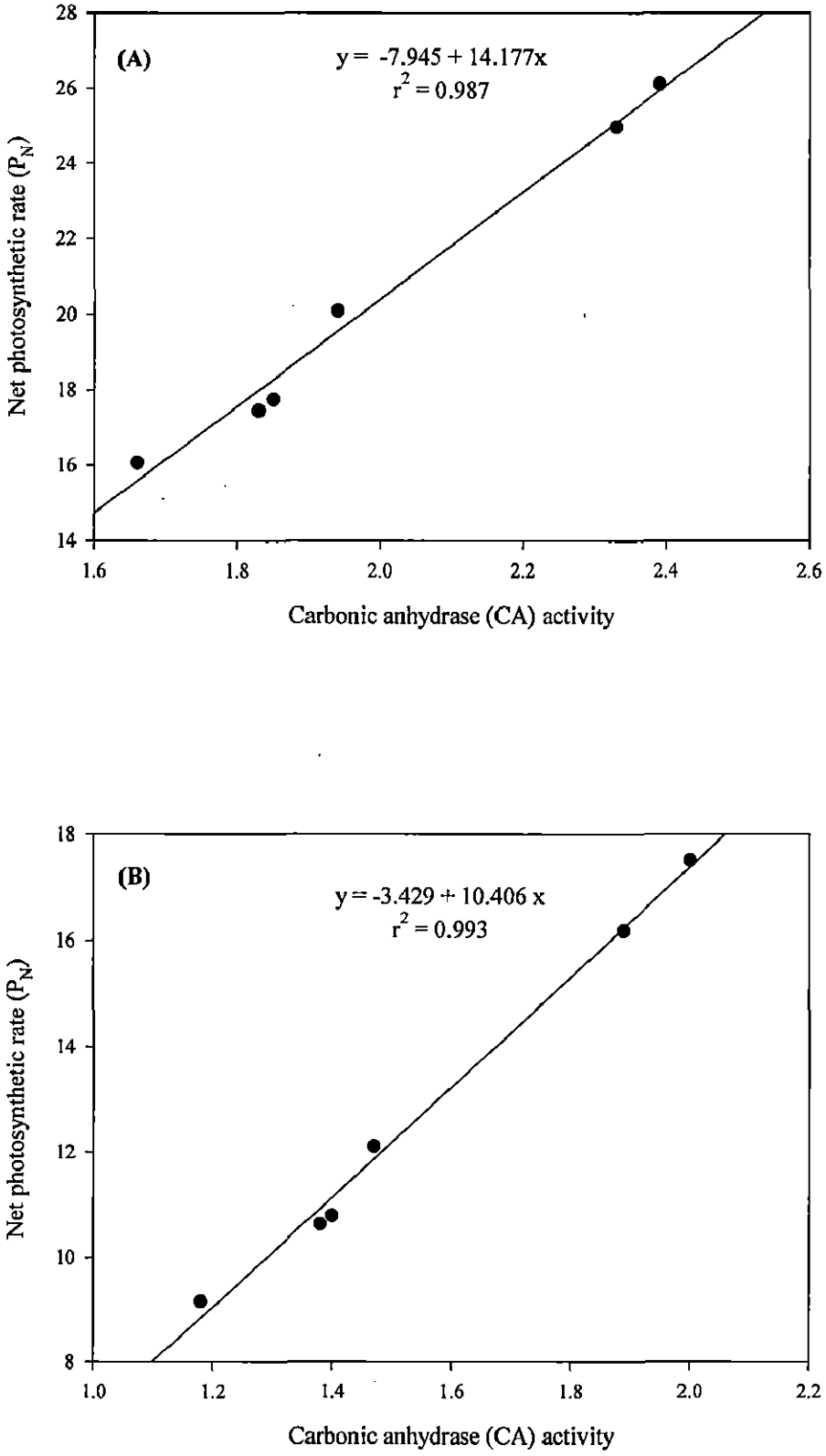


Figure 4: Correlation coefficient values between carbonic anhydrase (CA) activity and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 4)

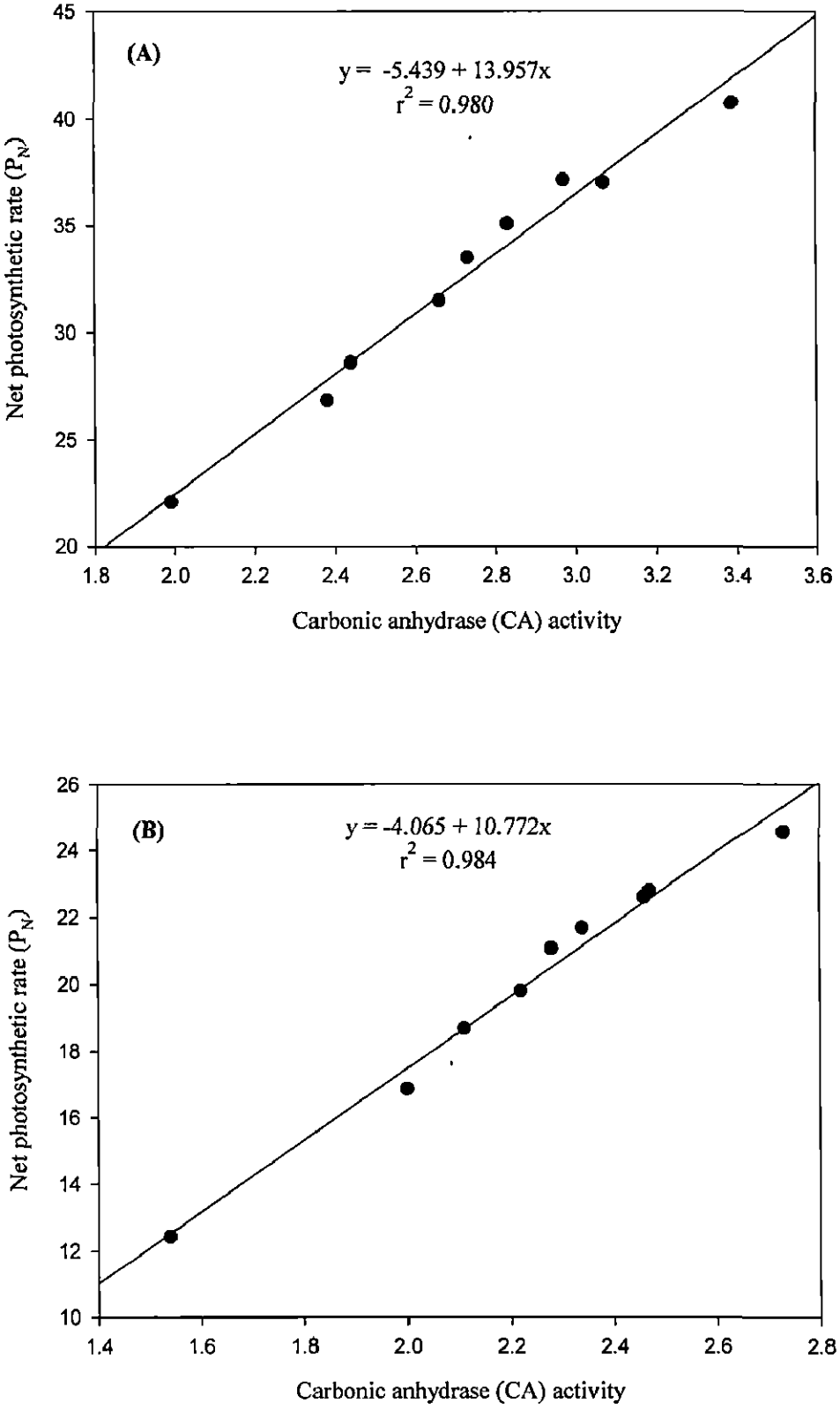


Figure 5: Correlation coefficient values between carbonic anhydrase (CA) activity and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 5)

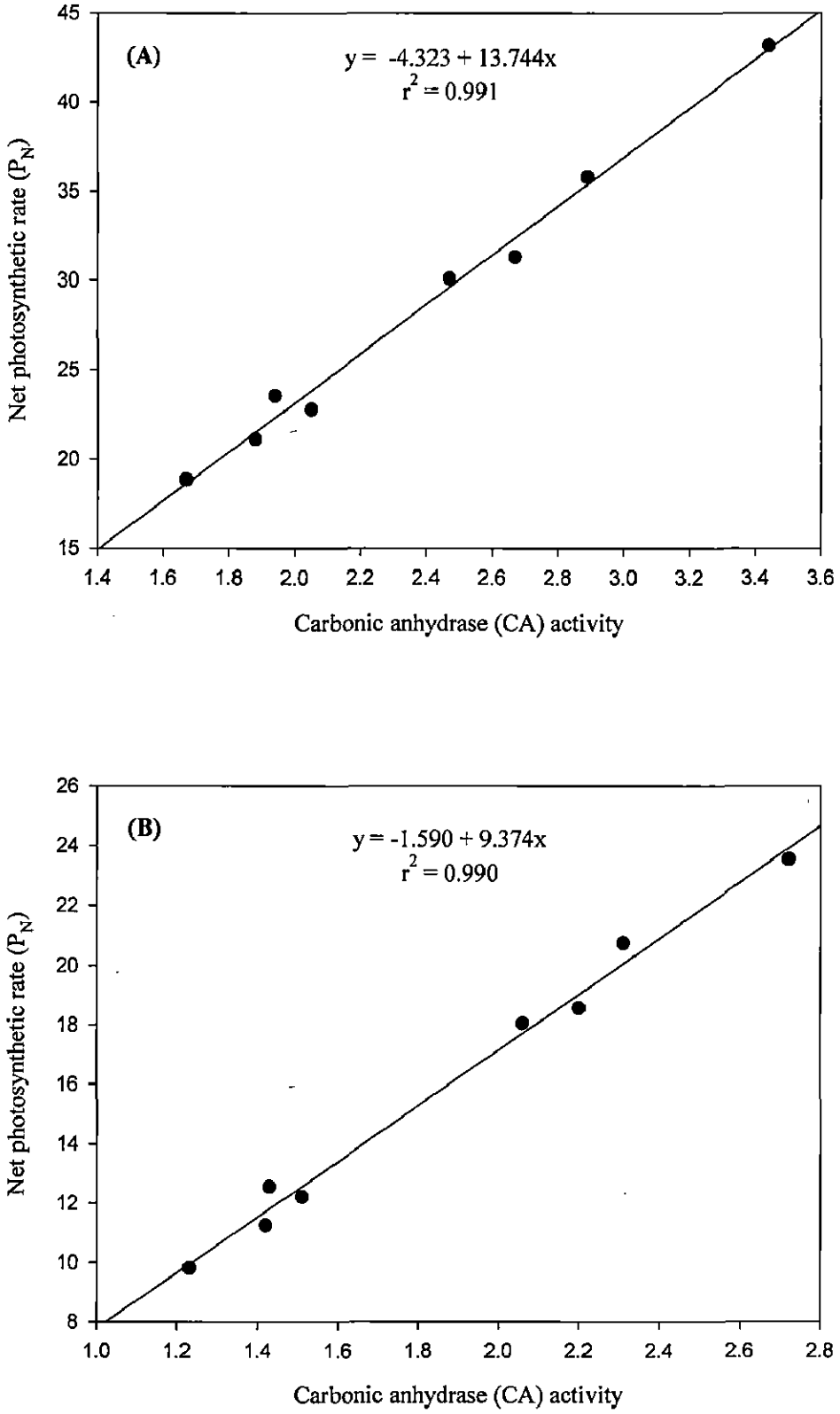


Figure 6: Correlation coefficient values between carbonic anhydrase (CA) activity and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 6)

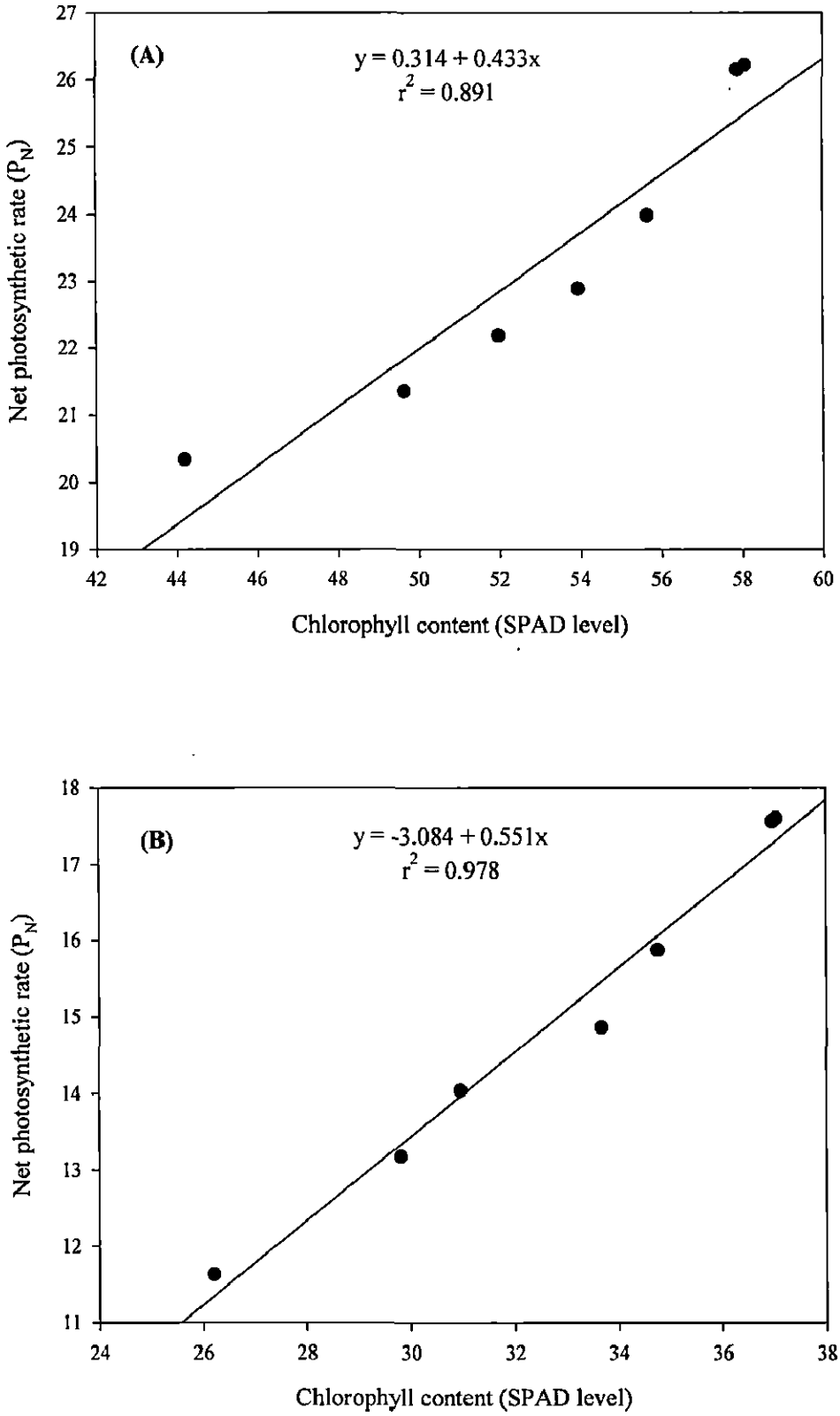
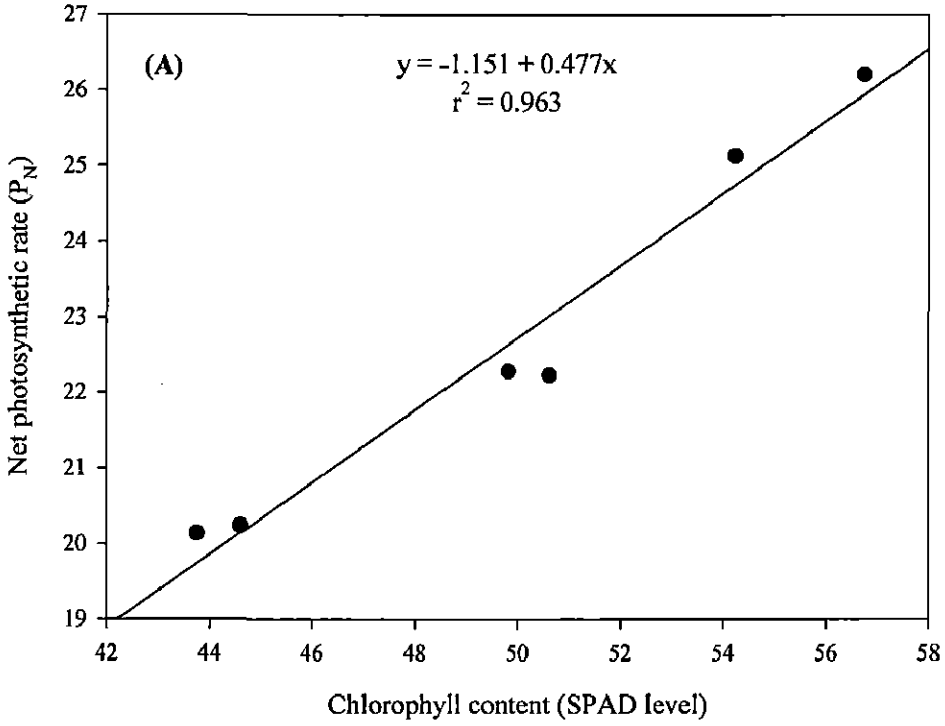


Figure 7: Correlation coefficient values between chlorophyll content (SPAD level) and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 1)



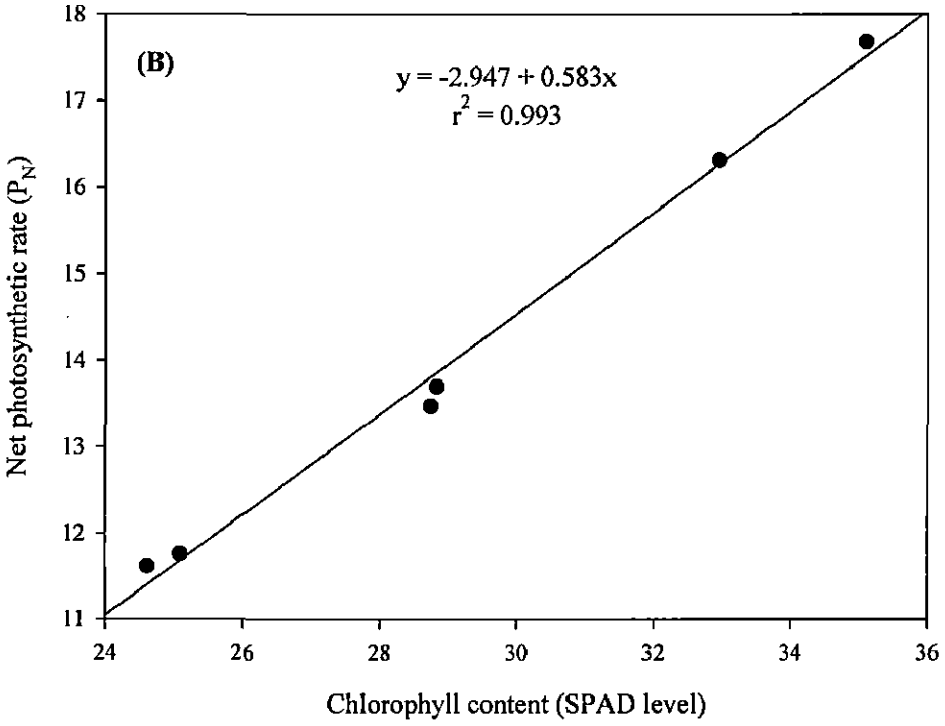
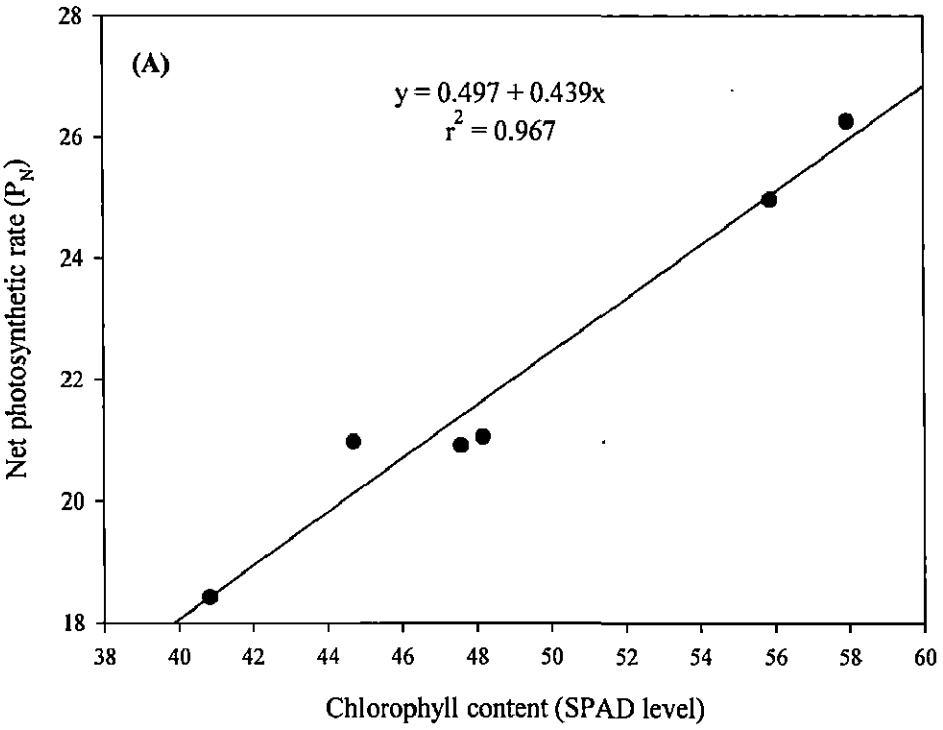


Figure 8: Correlation coefficient values between chlorophyll content (SPAD level) and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 2)



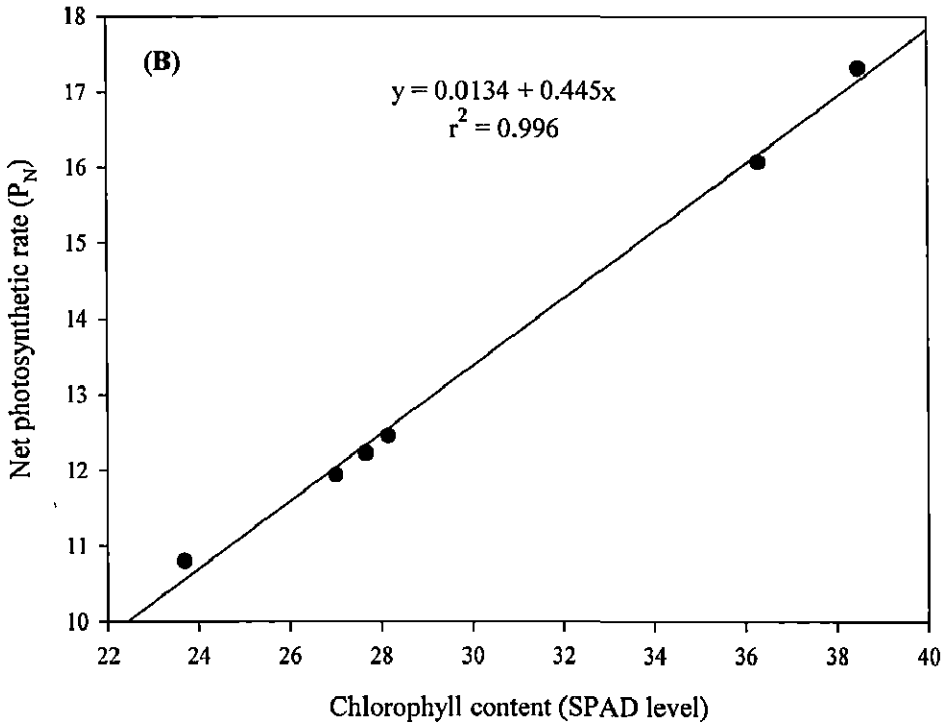


Figure 9: Correlation coefficient values between chlorophyll content (SPAD level) and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 3)

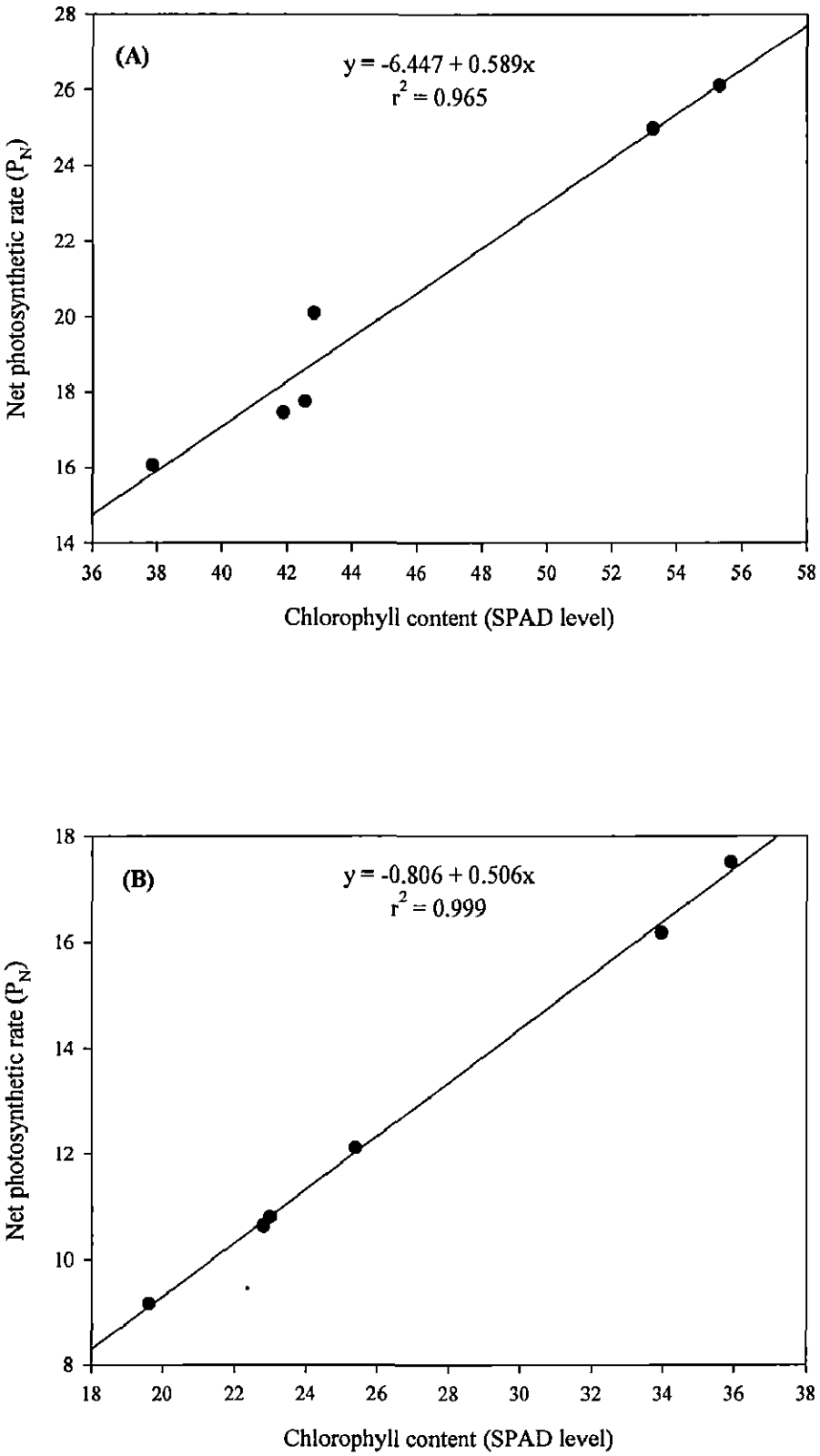
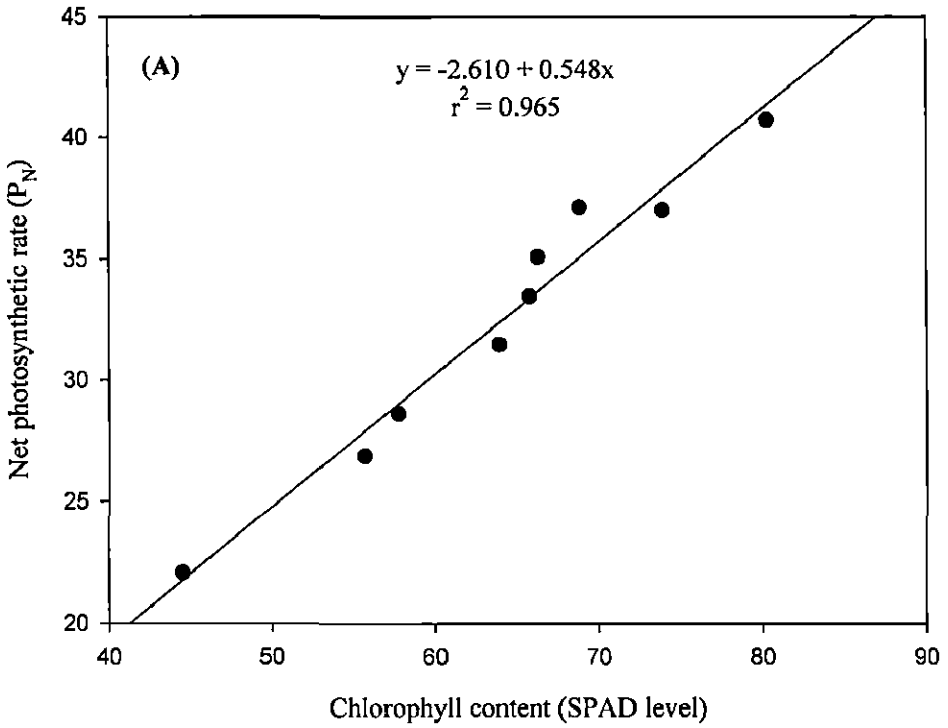


Figure 10: Correlation coefficient values between chlorophyll content (SPAD level) and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 4)



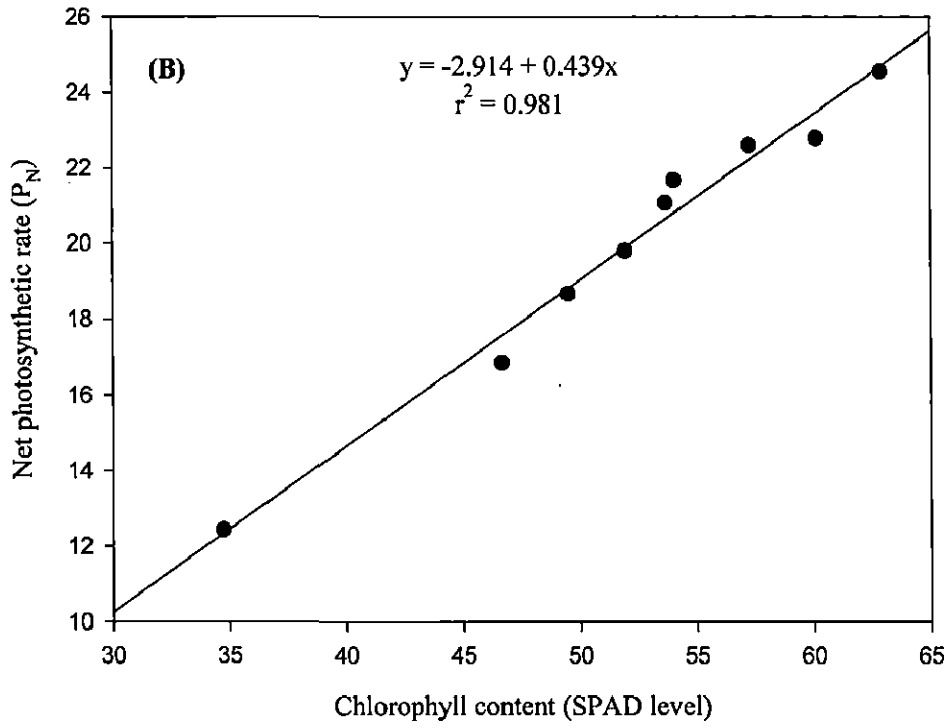
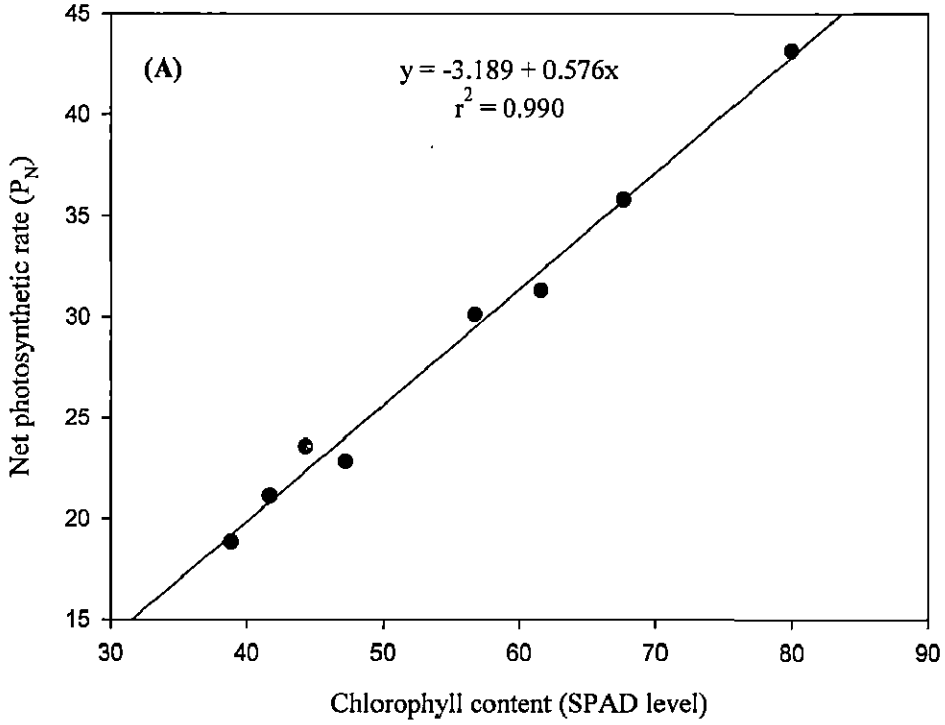


Figure 11: Correlation coefficient values between chlorophyll content (SPAD level) and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 5)



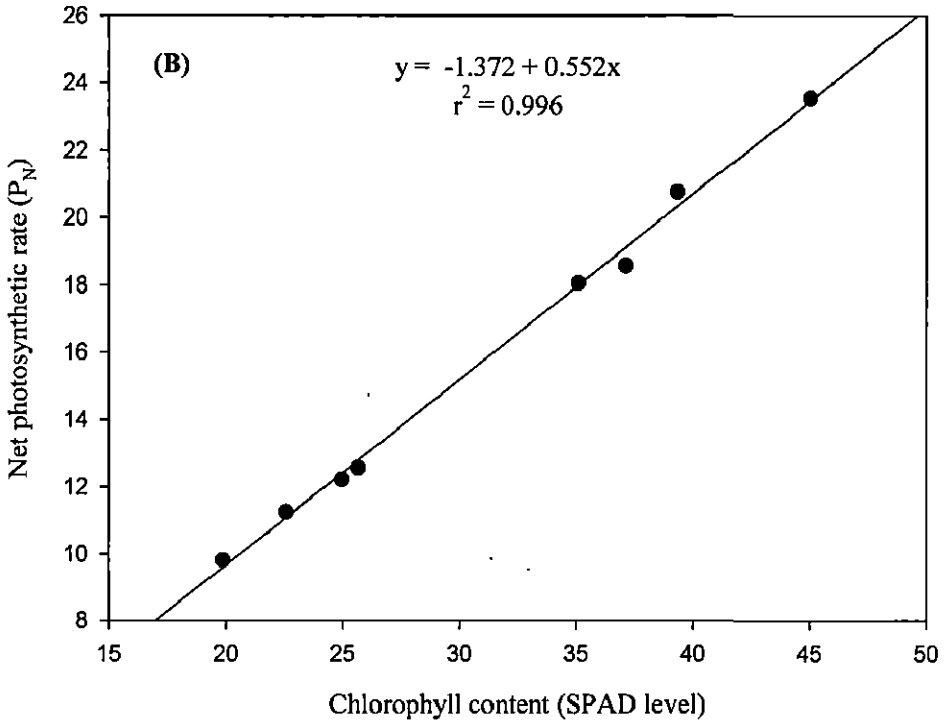


Figure 12: Correlation coefficient values between chlorophyll content (SPAD level) and net photosynthetic rate (P_N) in (A) Varuna and (B) Chapka Rohini (Experiment 6)

correlation between CA and P_N (Figures 5-6). BRs have also been assigned the role in the protection of photosynthetic apparatus (Hasan *et al.,* 2011), regulation of membrane anion channels (Zhang *et al.,* 2005), facilitation of stomatal conductance (Gudesblat *et al.,* 2012), improvement of Rubisco activity (Yu *et al.,* 2004; Anuradha and Rao, 2009; Hayat *et al.,* 2011b), enhancement of the efficiency of light harvesting system by elevating the level of chlorophyll (Tables 52 and 64), and CO_2 assimilation (Jiang *et al.,* 2012). Similar reasons have also been given by others to explain the increase in photosynthesis in the crops treated with BRs under various abiotic stresses (Alam *et al.,* 2007; Hasan *et al.,* 2008; Hayat *et al.,* 2010a, 2011a, b; Fariduddin *et al.,* 2011).

Maximum quantum yield of PSII (Fv/Fm) is the most frequently used fluorescence parameter (Bjorkman and Demming, 1987). The plants grown in the soil amended with varying levels of B had significantly lower values for the maximum quantum yield of PSII (Fv/Fm), more profoundly in the variety Chapka Rohini than in Varuna (Tables 7, 19, 31 and 43). This could have led to a reorganization of the light-harvesting complexes of PSII into aggregates of polypeptides containing chlorophylls and xanthophylls and to maximize the dissipation of thermal energy as well (Adams *et al.,* 2004). Evidences proved that the mechanisms which could be important, under excess supply of boron are (i) the increase in F_0, which indicates a damage in photochemistry, associated with a chronic loss of functional PSII reaction centers (Ottander *et al.*, 1995), and (ii) the lower Chlorophyll a/b ratio, suggests a preferential loss of reaction centers and core antenna proteins relative to light-harvesting proteins (Adams and Baker, 1998). The decrease in chlorophyll fluorescence attributed to oxidation of chlorophyll and chloroplastic membranes, which might have been exacerbated by the excess of B, as reported in orange (Papadakis *et al.*, 2004), hot pepper (Lee, 2006), apple rootstocks (Sotiropoulos *et al.,* 2006) and chick pea (Ardic *et al.,* 2009b). On the other hand, the application of NaCl alone decreased the photochemical efficiency (Tables 19, 31 and 43) which has been ascribed with the suppression of PSII activity (Mehta *et al.,* 2010).This suggests that the salt stress caused damage to PSII electron transport (Megdiche *et al.,* 2008) where it blocks the electron transfer from the primary acceptor plastoquinone (Q_A) to the secondary acceptor plastoquinone (Q_B) at the acceptor side of PSII which lead to the decrease in the values of Fv/Fm (Mehta *et al.,* 2010; Shu *et al.,* 2012). However, the simultaneous stress of NaCl and B caused a disorder in electron transport and photorespiration

(Frey *et al.,* 1998) resulting in a decrease of the maximum photochemical efficiency of PSII in *Viburnum tinus* (laurustinus) (Banon *et al.,* 2012). These findings support the conclusion that the combination of salinity and B toxicity hampered the photosynthetic system of mustard plants in the present study (Tables 19, 31 and 43). Moreover, NaCl and/or B-stressed plants sprayed with BRs showed higher values for Fv/Fm than (control), non-stressed plants (Tables 55 and 67) which indicates that the application of BRs helped in the protection of PSII against overexcitation, under abiotic stress that could have caused damage to the integrity of chloroplast thylakoid membrane (Ogweno *et al.,* 2008). Similarly, Yu *et al.* (2004) showed that treatment of EBL on cucumber plants led to an increase in the chlorophyll fluorescence. The other reports also showed that the application of BRs protects the quantum yield of PSII under chilling stress (Huang, 2005), salt stress (Houimli *et al.*, 2008), aluminium stress (Dong *et al.,* 2008), B stress (Yusuf *et al.,* 2011), and weak light stress (Wang *et al.,* 2010b).

Present study revealed that plants raised in the soil amended with B and NaCl alone or in combinations increased the leakage of ions through the membrane in both the varieties, compared to the control plants (Tables 7, 19, 31 and 43). Membrane damage might be caused by high H_2O_2 levels, which could have triggered the Haber-Weiss reaction, resulting in hydroxyl radical and thus resulted in lipid peroxidation (Mittler, 2002; Aftab *et al.,* 2012). Boron stress responsible for membrane damage by lipid peroxidation and H_2O_2 accumulation have been reported in *Malus domestica* and *Vitis vinifera* (Gunes *et al.,* 2006; Molassiotis *et al.,* 2006). It receives further support from Eraslan *et al.* (2007a) and Parida and Das (2005) who reported a significant increase in electrolyte leakage under B and salt stress, respectively. Moreover, the presence of NaCl in combination with B further increased the electrolyte leakage (Tables 19, 31 and 43) as a result of a greater damage to the membrane. This is in conformation with the findings of Alpaslan and Gunes (2001) in tomato and cucumber, and of Ismail (2004) in maize and sorghum. Contrary to this, BRs as a follow-up treatment to the stressed plants lowered the electrolyte leakage in B and/or NaCl stressed plants (Tables 55 and 67). These results are in conformity with those of Karlidag *et al.* (2011) who found that BR analogue, EBL facilitated the maintenance of membrane functions under salt stress condition in *Fragaria ananassa.* This facilitation by hormone could be attributed to the induction of antioxidant responses and elevated Ca uptake that protects the plant from the oxidative damage. Moreover,

BRs are reported to modify the membrane structure and stabilize it under stress conditions (Hamada, 1986).

Almost all the environmental stresses have been reported to induce the overproduction of reactive oxygen species (ROS) that may oxidize proteins, lipids and nucleic acids in the cells resulting in the abnormalities (Mullineaux and Baker, 2010; Sharma *et al.,* 2010). However, the plants possess efficient antioxidant systems for scavenging ROS, which protect the cells from destructive oxidative damage. As a part of this system, antioxidant enzymes represent an important line of defense against free radicals and oxidative stress (Andre *et al.,* 2010). In order to avoid this oxidative damage, plants enhance the level of endogenous antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), superoxide dismutase (SOD), glutathione reductase (GR) and non-enzymatic low molecular weight compounds like proline, glutathione, carotenoids, ascorbate, tocopherols etc. which control the level of ROS in plant tissues. In the present study mustard plants, grown in the soil amended with NaCl or B alone possessed enhanced activity of antioxidant enzymes like CAT, POX and SOD and an accumulation of proline (Tables 9-10, 21-22, 33-34 and 45-46). Various other crop plants like barley (Karabal *et al.,* 2003), tomato (Cervilla *et al.,* 2007), apple rootstocks (Molassiotis *et al.,* 2006; Sotiropoulos *et al.,* 2006), chickpea (Ardic *et al.,* 2009a, b), and mustard (Varshney *et al.,* 2015) are also reported to behave similarly under B stress. Similar observations under salt stress have also been reported earlier in *Brassica juncea* (Ahmad *et al.,* 2012; Hayat *et al.,* 2011a), *Vigna radiata* (Hayat *et al.,* 2010a) and *Beta vulgaris* (Farkhondeh *et al.,* 2012). Stress induced consequences in plants are protected by multifunctional metabolites, like proline (Szabados and Savoure, 2010). Proline under stress conditions, acts as a scavenger of free radicals and stabilizer of plasma membrane and of some macromolecules, like DNA (Jain *et al.,* 2001; Filippou *et al.,* 2014) thereby, protecting the plants under extreme conditions. The synthesis of proline is a gene-regulated process that involves its activation to induce proline biosynthesis and downregulation of those involved in its degradation (Sumithra and Reddy, 2004), under stress conditions. Salt-induced accumulation of proline could have been due to the increased rate of hydrolysis of proteins (Irigoyen *et al.,* 1992) as the protein synthesizing machinery is diverted towards the proline accumulation (Claussen, 2005). Secondly, an enhanced level of proline could be due to its slower rate of degradation (Kiyosue *et al.,* 1996). Recently, Hayat *et al.* (2014) reported

positive impact of proline on the antioxidant system and photosynthesis to secure plant growth, under stressed conditions. Moreover, present study has also shown that salt and B together further enhanced the activity of CAT, POX and SOD and proline content, leading to further increase in oxidative stress (Tables 21-22, 33-34 and 45-46). These observations get additional support from Lee (2006) who found that the combination of salt and B stress increased the activity of antioxidant enzymes such as, peroxidase, superoxide dismutase, ascorbate peroxidase, glutathione reductase and proline content in hot pepper plants. Furthermore, the increase in proline accumulation in our case is well supported by the findings of Eraslan *et al.* (2007b) in *Daucus carota*. The stress of salt plus B causes a physiological disorder that could have accelerated the uptake of potentially toxic ions and increased water deficit leading to increased oxidative stress which restricted plant growth (Lee, 2006). Therefore, findings indicated that the dual effect of NaCl and B toxicity created a hostile condition for mustard plants to grow. Out of the two selected varieties, Varuna possessed higher proline content and the activity of CAT, POX and SOD enzymes than Chapka Rohini. Such varietal responses are being assigned due to better ability of Varuna to cope with stress due to enhanced antioxidant apparatus.

On the other hand, application of BR analogues (HBL or EBL) to the foliage of stress-free and stressed plants improved their activity of antioxidant enzymes and the level of proline (Tables 57-58 and 69-70). The increased level of proline induced by BRs (Ozdemir *et al.*, 2004) could have checked the cellular uptake of salt ions in the cytoplasm thereby working as a compatible osmolyte (Flores and Galston, 1984). The specific genes are said to be involved in the regulation of proline level, under stress in *Arabidopsis* (Rentsch *et al.*, 1996) and tomato (Schwacke *et al.*, 1999). Moreover, the enhancement in the activity of antioxidative enzymes by BRs is a gene regulated phenomenon, like the expression of POX-encoding genes *ATP2* and *ATP24a* (Goda *et al.*, 2002). Moreover, on the basis of molecular, physiological and genetic approaches it could be stated that this elevation in antioxidant enzymes by BRs was the consequence of enhanced expression of *DET2* gene, which improved the tolerance capabilities to oxidative stress in *Arabidopsis* (Cao *et al.*, 2005). In addition, BRs confer the tolerance to oxidative stress through the increased activity of NADPH oxidase enzyme and the elevated level of H_2O_2 in the apoplast (Xia *et al.*, 2009). Brassinosteroids perception by receptors activates the plasma membrane-bound NADPH oxidase which results in the elevation of the level of H_2O_2 to initiate protein

phosphorylation cascade (Xia *et al.,* 2009). The H_2O_2 mediates the transcriptional induction of defense or antioxidant genes. Transcription factors may be activated via a phosphorylation cascade by MAPKs (Mitogen-activated protein kinases). Finally, the products of target genes participate directly in cellular protection against the stress (Xia *et al.,* 2009). Similarly, the application of HBL increased the activity of POX and SOD enzymes and also decreased lipid peroxidation of the membrane in rice (Chen *et al.,* 2007). Therefore, the enhanced activity of antioxidant enzymes in association with proline accumulation, under stress conditions suggest a potential role for BRs in the amelioration of oxidative stress generated by B alone or in combination with NaCl in mustard plants.

In the present study, mustard plants exposed to varied levels of, soil amended, B and NaCl, alone or in combination showed significantly reduced growth traits, reflected in terms of loss in length, fresh and dry mass of shoot and root as well as leaf area (Tables 1-4, 13-16, 25-28 and 37-40).The different concentrations (20, 30, 40, 50 or 60 mg kg^{-1} soil) of B suppressed plant growth in a concentration dependent manner in both the varieties (Varuna and Chapka Rohini) (Tables 1-4). Boron suppressed plant growth could be due to its toxic effects on root cell division, cell wall expansion, chlorophyll content, and photosynthetic rate (Nable *et al.,* 1997; Herrera-Rodriguez *et al.,* 2010; Esim *et al.,* 2012). This could partly be due to the retardation in normal biosynthetic activities, energy transduction, protein synthesis and inhibition of many important cellular processes (Reid *et al.,* 2004) and altered activities of antioxidant enzymes (Karabal *et al.,* 2003) cumulatively leading to the impairment in plant growth. Our results get further support from the earlier observations in tomato (Alpaslan and Gunes, 2001; Cervilla *et al.,* 2009), apple rootstocks (Mouhtaridou *et al.,* 2004), mustard (Javid *et al.,* 2014), wheat (Coskun *et al.,* 2014) and watermelon (Hamurcu *et al.,* 2015). Moreover, the toxicity generated by B in roots was comparatively more prominent than in the shoots (Tables 1-3). Root growth was severely inhibited under B stress that has also been reported in earlier studies (Reid *et al.,* 2004; Ardic *et al.,* 2009a; Karabal *et al.,* 2003). It might be due to more accumulation of B in roots that results in reduced root elongation and lateral root development (Kaur *et al.,* 2006). Out of the two varieties tested, Varuna was more tolerant to B stress than Chapa Rohini. This varied growth response of two varieties of mustard could possibly be due to differential regulation of the processes related to the growth at their genetic, biochemical and physiological levels.

Furthermore, the improved tolerance of Varuna to B could be related to higher exclusion of B at the root level, mediated by reduced permeability of membrane lipids and/or presence of carriers (BOR and NIP) essential for exclusion (Miwa *et al.*, 2007).

The varying levels (2.8, 4.2 or 5.6 dSm^{-1}) of salt are believed to decline growth through the inhibition of cell division and cell elongation (Pitann *et al.*, 2009) which is mainly due to osmotic effects, ion toxicities and mineral disturbances in plants (Tester and Devenport, 2003) which in turn impaired plant growth and biomass production (Tables 13-16, 25-28 and 37-40). Salinity restricts cell expansion by decreasing the uptake of water or osmolytes, alterations in turgor, and cell wall properties (Taleisnik *et al.*, 2009). It also affects availability and transport of mineral nutrients, essential for plant growth and development. Nutrient imbalances or deficiencies are generated due to the competition of Na^+ and Cl^- with mineral nutrients like K^+ and Ca^{2+} under saline conditions which reduce plant growth due to specific ion toxicities (e.g. Na^+ and Cl^-) and ionic imbalances acting on biophysical and/or metabolic components of plants (Grattan and Grieves, 1999). Increase in NaCl level has been reported to enhance the concentration of Na and Cl, which leads to a decrease in the essential mineral elements like N, P, K, Ca and Mg (Abd El-Wahab, 2006; Ashraf and Orooj, 2006; Baghalian *et al.*, 2008; Abd El-Azim and Ahmed, 2009; Taffouo *et al.*, 2010). Similar impacts of salt stress on the growth of *Brassica juncea* (Wani *et al.*, 2013), *Solanum lycopersicum* (Zribi *et al.*, 2009), *Helianthus annuus* (Akram and Ashraf, 2011) have also been reported earlier. However, the earlier findings regarding the effects of combined salinity and B toxicity are contradictory (Masood *et al.*, 2012). Moreover, in the present study, the interactive stress of salt with excess B had additive, negative effects on growth and development of mustard plants (Tables 13-16, 25-28 and 37-40). This in agreement with Apostol *et al.* (2002) who reported that jack pine treated with 2mM B showed reduced root growth. Moreover, in the presence of 60 mM NaCl and Na_2SO_4 treatments, and high B, the plants exhibited further loss in growth. Salinity aggravated B toxicity symptoms have been reported in a variety of plant species which is accompanied by an increase B concentrations in shoot (Grieve and Poss, 2000).

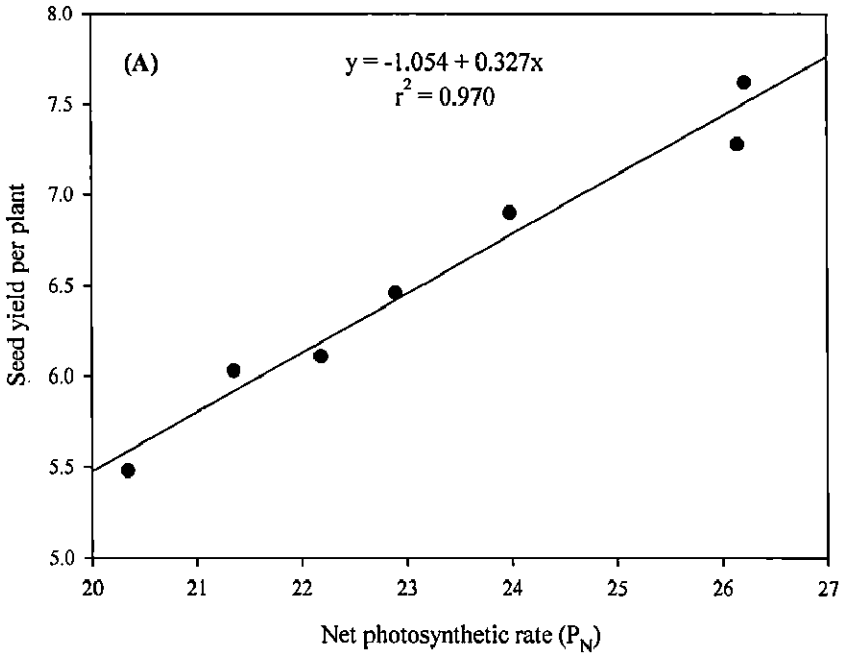
The exogenous application of BRs alone or as a follow-up treatment to the B and/ or salt-stressed plants improved the value of growth biomarkers (length, fresh and dry mass of root and shoot and leaf area) in both the varieties of mustard (Tables 49-52 and 61-64). BRs are involved in the transcription of cyclin-D3 protein, a key

regulator of cell cycle in *Arabidopsis* (Ashraf *et al.,* 2010), which could be assigned as the main role for BR activated cell division and cellular enlargement (Clouse and Sasse, 1998; Bajguz and Tretyn, 2003) leading to improved plant growth. Aquea *et al.* (2012) demonstrated that the presence of higher concentration of B regulate cell cycle in root tissue and cause alterations in the expression level of key cell cycle gene (*CycD3;1*). However, the application of EBL alone or in combination with B enhanced the expression level of *CycD3;1* gene in the root tissue (Surgun and Burun 2015). Thus, this promotive effect of BRs on cell cycle progression and cell division is attained through the enhancement in the expression of *CycD3;1* gene (Surgun and Burun, 2015).

Moreover, one of the genes of this group is *TCH4* gene that encodes xyloglucan endotransglucosylase/hydrolase (XTHs) protein in *Arabidopsis thaliana*. Xu *et al.* (1995) revealed that the expression of *TCH4* gene encoding XTHs protein in *Arabidopsis thaliana* is regulated rapidly in response to environmental stress and changes in the expression level of *TCH4* gene directly causing modifications in cell wall properties and their structure. Moreover, Surgun and Burun (2015) reported that EBL treatment alone or in combination with B, increased the expression levels of *TCH4* gene in root and leaf tissue. Thus, it has been concluded that regulation of the expressions of gene that encodes cell cycle (*CycD3;1*) and cell-wall modifying enzymes (*TCH4*) by the application of BRs is important for regulating the morphogenetic response of plants under stress-free and stressed conditions (Tables 49-52 and 61-64). Results of the present study are further corroborated by other studies where BRs improved the growth, like that in *Vigna radiata* under B stress (Yusuf *et al.,* 2011), *Brassica juncea* under Ni stress (Alam *et al.,* 2007), *Lycopersicon esculentum* under Cd stress (Hayat *et al.,* 2010c), *Cucumis sativus* under Cu stress (Fariduddin *et al.,* 2013) *Solanum melongena* and *Vigna sinensis* under salt stress (Wu *et al.,* 2012; El Mashad and Mohamed, 2012). Thus, it looked quite convincing that how application of BRs protects the plants from the damage generated from B and/or salt stress.

The yield characteristics (pods number per plant, seeds number per plant, 100 seed mass and seed yield per plant) significantly decreased in response to soil applied B and/or NaCl (Tables 11-12, 23-24, 35-36 and 47-48).The loss in yield could primarily be attributed to poor vegetative plant growth (Tables 1-4, 13-16, 25-28 and 37-40) resulting largely from the lower pace of CO_2-reduction (Tables 53 and 65 and

Chen *et al.,* 2009) and unfavourable nature of conducting pathway (Aldesuquy and Ibrahim, 2001) where the leaves start behaving as sinks rather than source. This causes inhibition of assimilate movement towards the developing reproductive organs, to make them weak and less productive (Arbona *et al.,* 2005). B stress limits plant growth or damages the photosynthetic system which lead to serious physiological implications, such as the disruption of auxin biosynthesis, leading to reduction in crop yield (Nable *et al.,* 1997; Le, 2006). It may also be due the inhibition of root development, limited uptake of essential nutrients and water, resulting necrosis on the leaves, thus reduced the rate of photosynthesis, and finally affecting fruit and seed development (Reid, 2007b). The plant growth and yield are also reduced due to salinity at the soil level that favours excess uptake of potentially toxic ions (Gupta *et al.,* 1985). Present study reported that the interaction of NaCl and excess B had an additive negative effect which further limited the yield of mustard plants (Tables 23-24, 35-36 and 47-48). Similar observations of salinity and excess B on the yield and yield component in wheat (Manchanda and Sharma, 1991; Holloway and Alston, 1992; Greive and Poss, 2000; Wimmer *et al.,* 2003), cucumber (Alpaslan and Gunes, 2001), tomato (Ben-Gal Shani, 2002) and maize and sorghum plants (Ismail, 2004) have also been reported by others. However, BRs applied to the foliage of stressed and stress-free plants improved all the yield characteristics (Tables 59-60 and 71-72) which could be an expression of higher rate of photosynthesis (Tables 53 and 65) that facilitated the availability of more carbohydrates for metabolism and export to the sink (Bajguz and Asami, 2005) for their healthy growth. Moreover, directed transportation of photosynthates to the sink in association with induced expression of genes encoding enzymes of carbohydrate metabolism (Roitsch, 1999) and retarding the process of senescence before and/or after pollination (Iwahori *et al.,* 1990) could have naturally helped the plants in extending the duration of photosynthetically active sites and also to prevent the premature loss of flowers and fruits leading to improved seed yield. The observations get additional support from Gomes *et al.* (2003) who noted higher biological yield in passion fruit, correlating it with higher photosynthetic carbon assimilation under BRs. It is strengthened by the establishment of a significantly positive correlation between net photosynthetic rate and seed yield per plant under stress-free as well as hormone plus stressed conditions, at harvest (Figures 13-18). BRs also favored yield characteristics in *Vigna radiata* (Fariduddin, 2002),



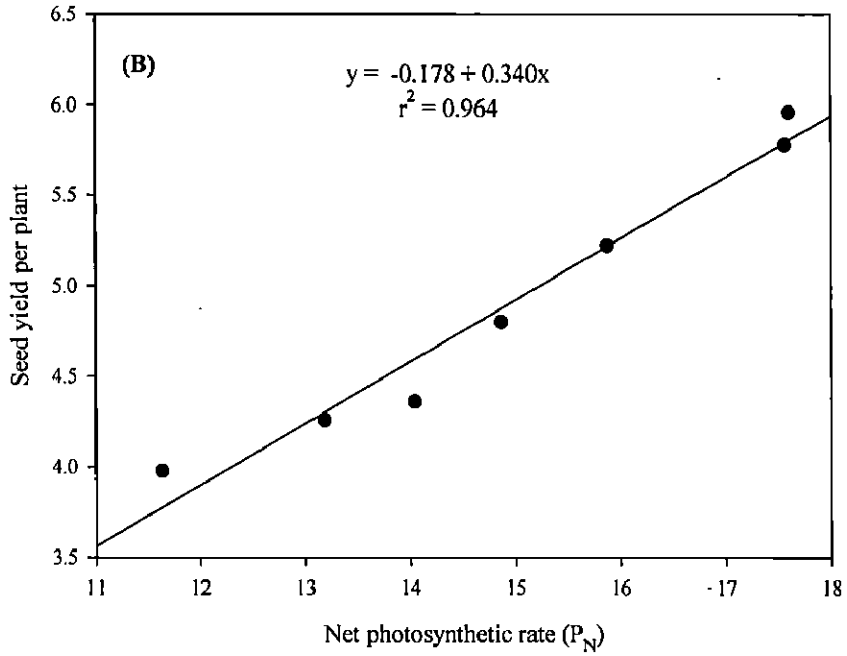
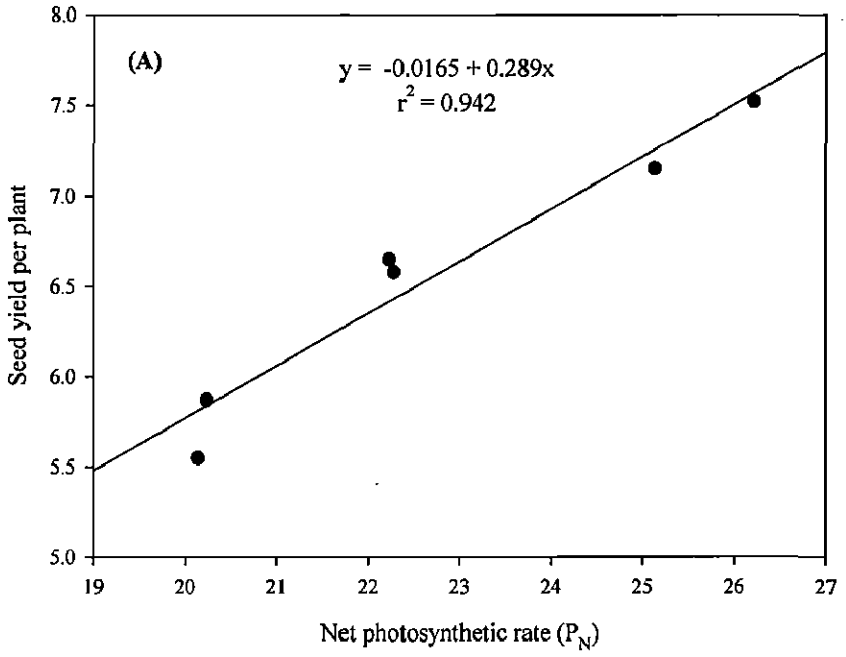


Figure 13: Correlation coefficient values between net photosynthetic rate (P_N) and seed yield per plant in (A) Varuna and (B) Chapka Rohini (Experiment 1)



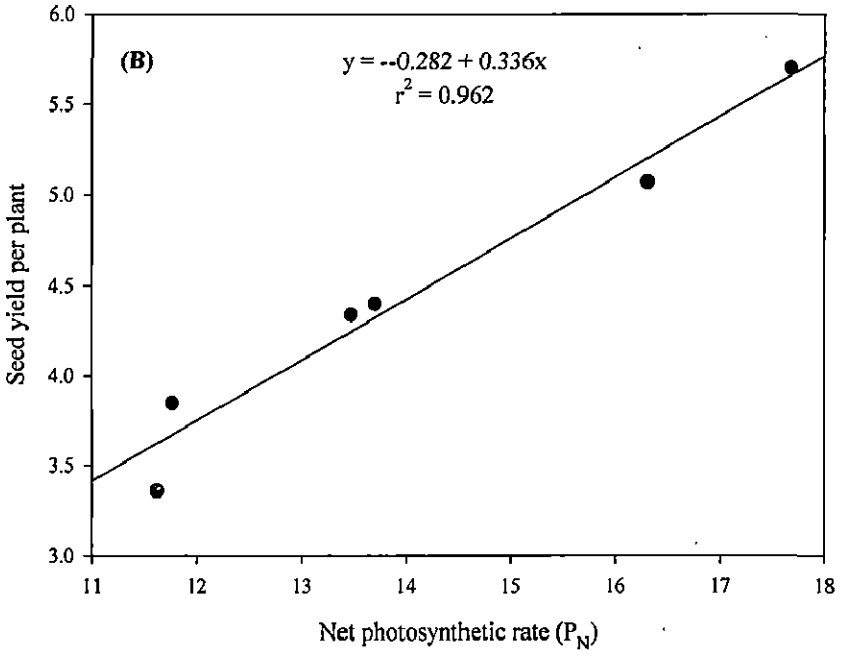
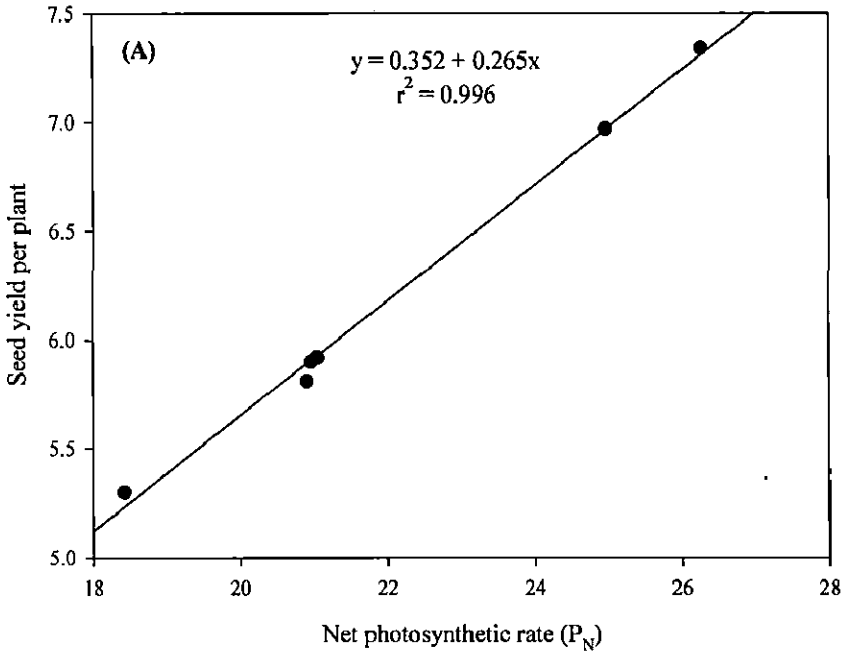


Figure 14: Correlation coefficient values between net photosynthetic rate (P_N) and seed yield per plant in (A) Varuna and (B) Chapka Rohini (Experiment 1)



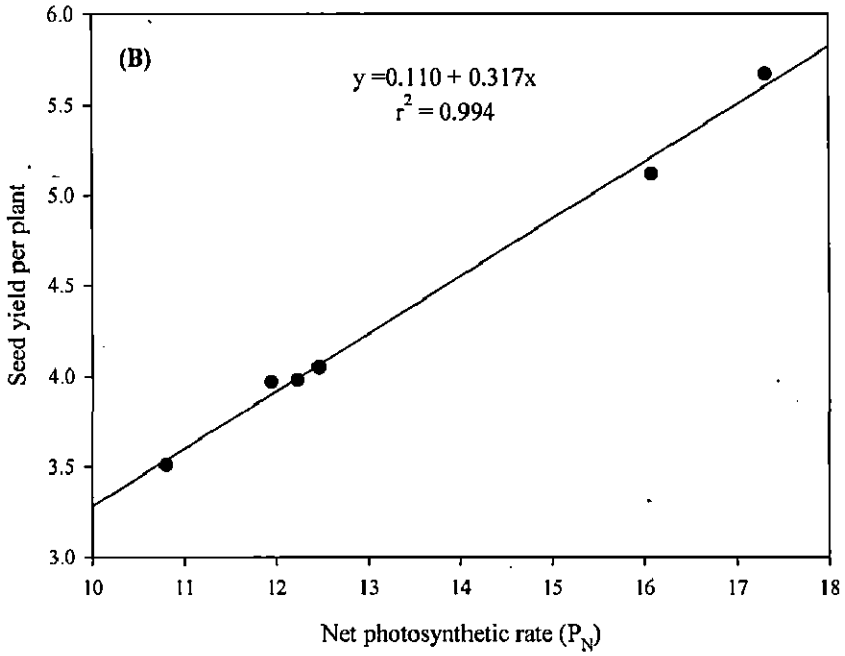
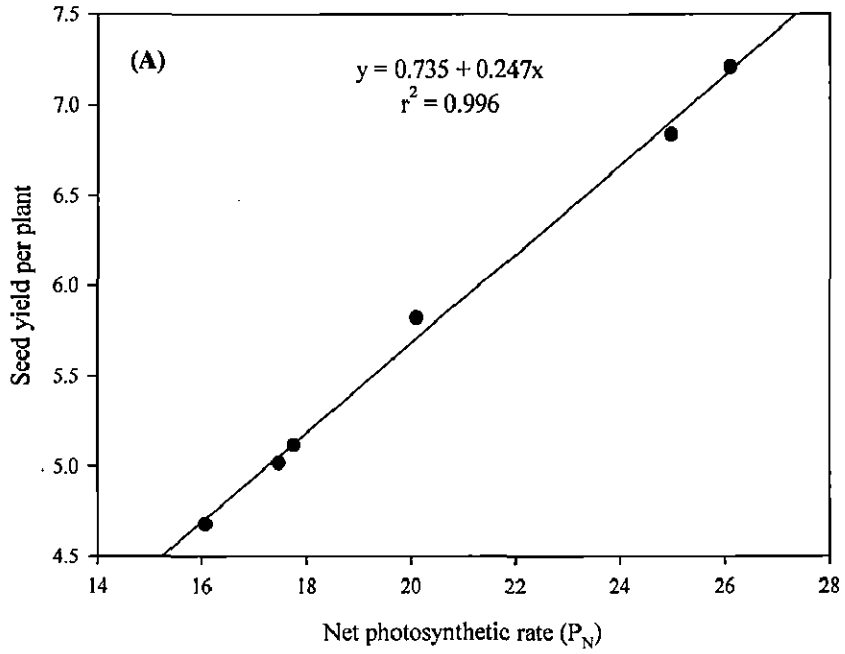


Figure 15: Correlation coefficient values between net photosynthetic rate (P_N) and seed yield per plant in (A) Varuna and (B) Chapka Rohini (Experiment 3)



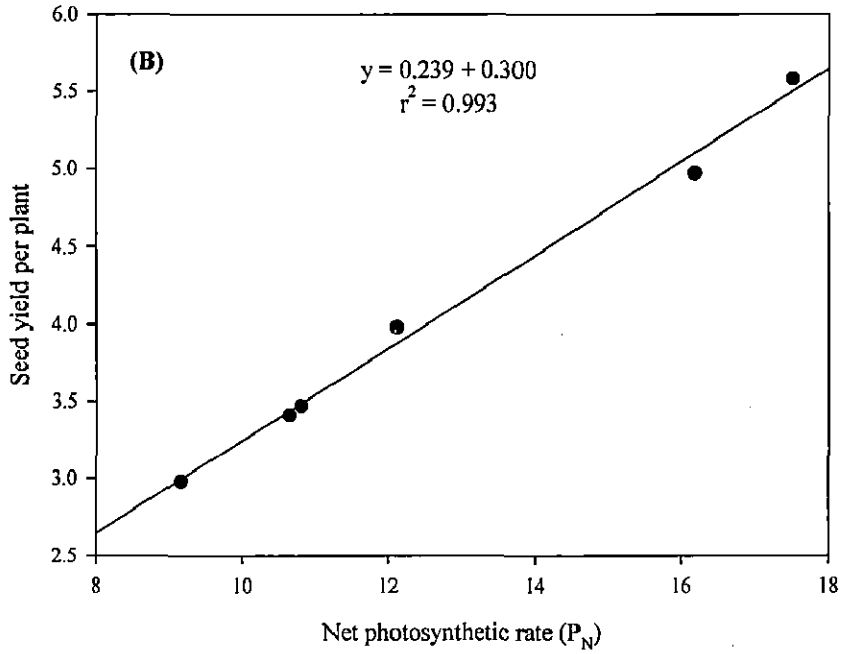
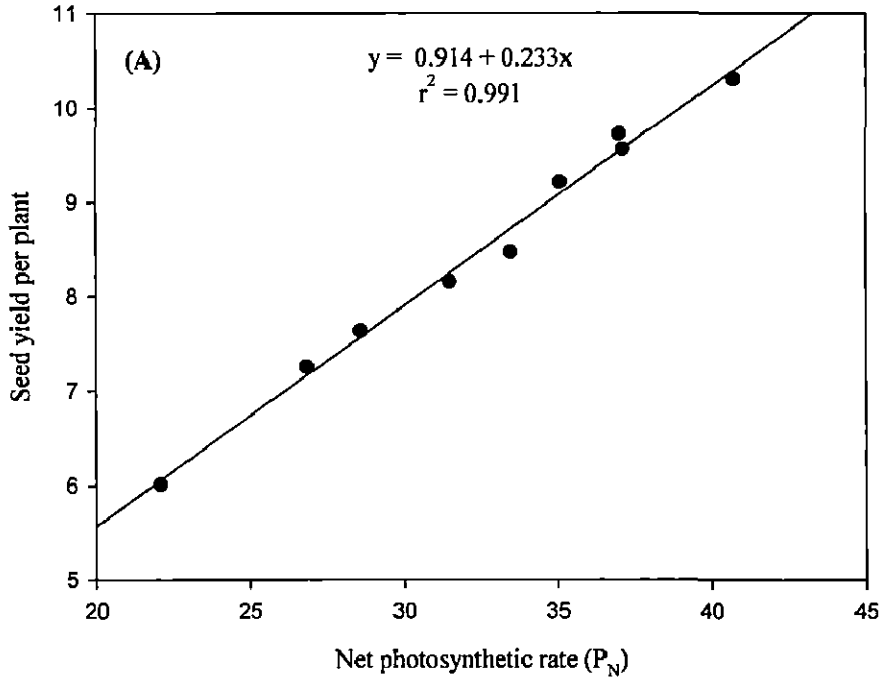


Figure 16: Correlation coefficient values between net photosynthetic rate (P_N) and seed yield per plant in (A) Varuna and (B) Chapka Rohini (Experiment 4)



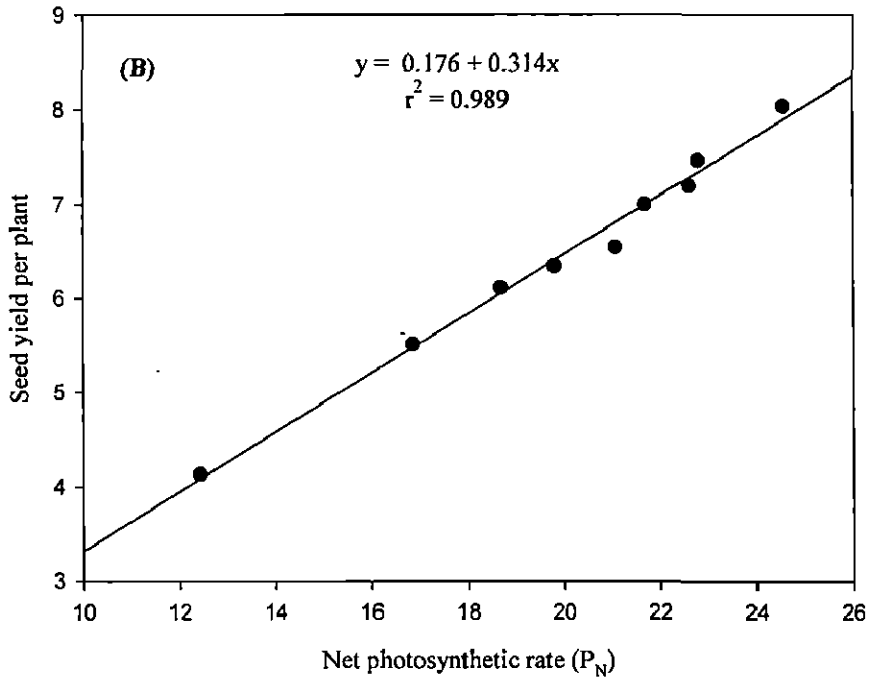
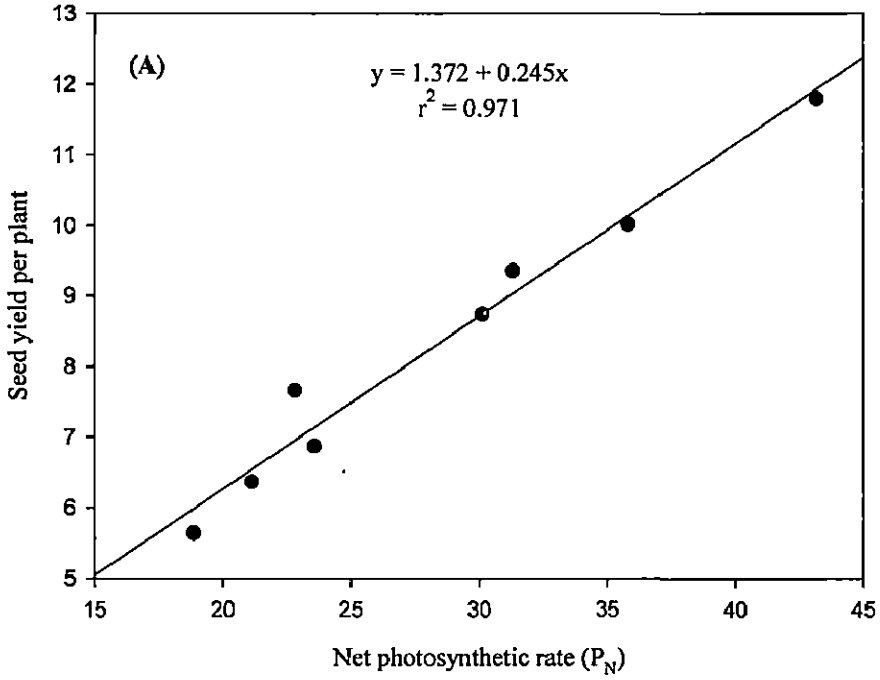


Figure 17: Correlation coefficient values between net photosynthetic rate (P_N) and seed yield per plant in (A) Varuna and (B) Chapka Rohini (Experiment 5)



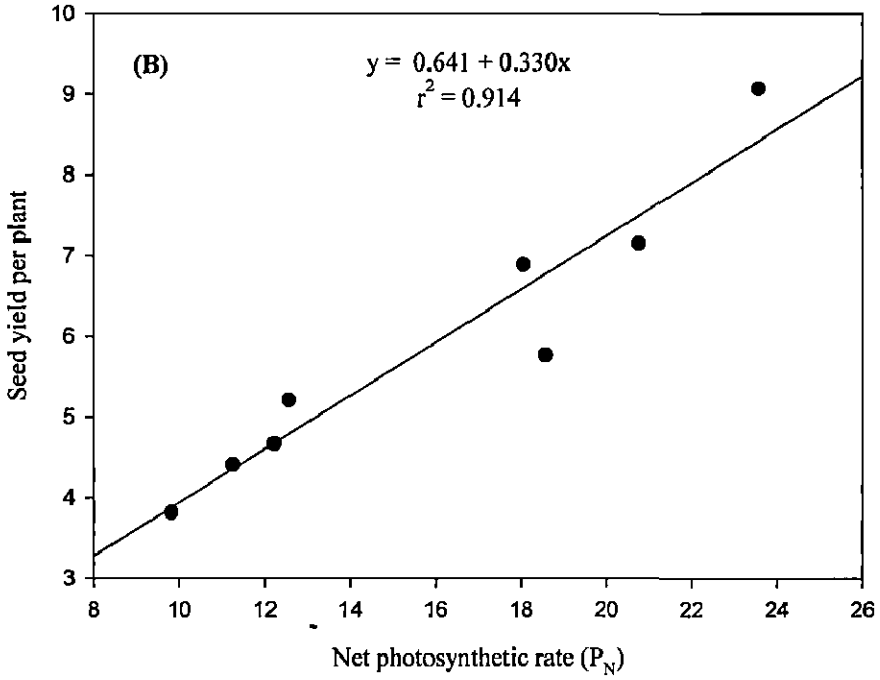


Figure 18: Correlation coefficient values between net photosynthetic rate (P_N) and seed yield per plant in (A) Varuna and (B) Chapka Rohini (Experiment 6)

Brassica juncea (Hayat *et al.*, 2000; Hayat *et al.*, 2001a) and *Lens culinaris* (Hayat and Ahmad, 2003).

Moreover, out of the two BR analogues (HBL/EBL), EBL proved more effective in improving the values of most of the morphological, physiological and biochemical parameters in the presence as well as in the absence of B-induced stress (Tables 49-60). This superiority of EBL over HBL may be because of differences in the structure and stability of these two analogues (Khripach *et al.*, 2003). S-oriented alkyl (methyl or ethyl) group at C-24 of side chain is present in almost all BRs while EBL and CS (another BR analogue) being exceptions which carry R-oriented alkyl group on the side chain of the steroid nucleus (Plate VI). It is, therefore, inferred that the attachment of EBL at its receptor on the plasma membrane leads to more distorted three dimensional structure conformational state as compared to HBL. This thermodynamically acquired new stable state of EBL seems to be more actively involved in triggering wide array of cascades, more efficiently than HBL. However, further study is warranted to know about the transcription factors that are involved in BKI-1 dissociation with BAK-1 to avail the binding in BRI-1 at membrane (Swaczynova *et al.*, 2007; Hategan *et al.*, 2010; Codreanu and Russinova, 2010). Thus, the exogenous application of EBL completely or partially neutralized the deleterious effects of salinity in combination with B. However, more study is needed at molecular level to disclose the crosstalk of BRs with other phytohormones in providing complete tolerance to salt and B stress. An outline summary of the effect of BRs on the B and/or salinity induced changes in plants is shown in plate VII. Out of the two cultivars tested, Varuna was more tolerant to NaCl and/or B stress than Chapka Rohini. This varied growth response of the two varieties of mustard could possibly be due to differential regulation of the processes related to growth at their genetic, biochemical and physiological levels.

CONCLUSION

The present study has revealed that:-

1. Out of the six concentrations (10, 20, 30, 40, 50 or 60 mg kg^{-1} of soil) of B, 20 mg kg^{-1} was found least toxic whereas, 60 mg kg^{-1} generated maximum toxicity in *Brassica juncea*.
2. Out of the two varieties (Varuna and Chapka Rohini) tested, Varuna was more tolerant than Chapka Rohini to B stress.

3. Both the varieties experienced a significant reduction in all the morphological, photosynthetic, biochemical and yield parameters, with the increasing level (20, 30, 40, 50 or 60 mg kg^{-1}) of B amended into the soil.
4. Lowest concentration of B (20 mg kg^{-1}) generated minimum toxicity whereas, highest concentration of B (60 mg kg^{-1}) triggered maximum toxicity in combination with all the three levels of NaCl (2.8, 4.2 or 5.6 dSm^{-1}).
5. Of the various combinations of NaCl and B tested, the higher concentration of NaCl (5.6 dSm^{-1}) in combination with B (60 mg kg^{-1}) generated maximum toxicity and lowered the values of the parameters expressing growth, physiological, biochemical and yield characteristics, in both the varieties.
6. All the morphological biomarkers and photosynthetic traits along with various biochemical parameters increased significantly in the plants treated with either of the brassinosteroid analogues (HBL or EBL) under both stress-free and stressed conditions.
7. Toxic effects generated by the two levels (20 or 60 mg kg^{-1}) of B were neutralized by the follow up treatment with either of the BR analogues (HBL or EBL), more efficiently in Varuna than Chapka Rohini.
8. Out of the two BR analogues tested, EBL excelled in its effect over HBL.
9. Application of EBL (10^{-8} M) increased the growth rate, activity of the enzymes (carbonic anhydrase and nitrate reductase), the photosynthetic attributes and yield characteristics as a follow up treatment to the plants, exposed to NaCl in combination with B.
10. Activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) and proline accumulation were enhanced further by the application of EBL (10^{-8} M) as a follow-up treatment to the NaCl plus B stressed plants.
11. The exogenous application of EBL (10^{-8} M), as a follow-up treatment proved most potent salt and excess boron stress alleviator by enhancing the level of antioxidant system and osmolyte (proline), manifested as improved growth, higher rate of photosynthesis, decreased electrolyte leakage and increased biological yield of mustard plants.

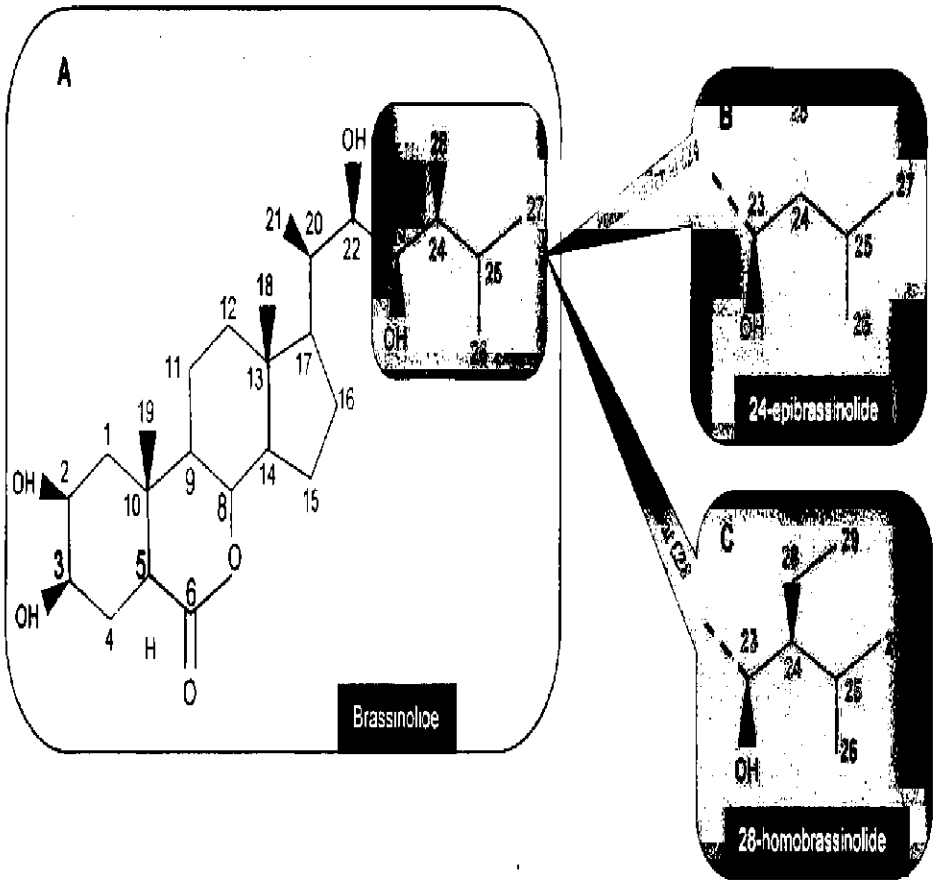


Plate VI: Structural difference between (A) Brassinolide (B) 24-epibrassinolide and (C) 28-homobrassinolide

The two important BRs, 24-EBL (B) and 28-HBL (C), differ from BL (A) by the substituent at C-24 or b its configuration (R or S orientation) of the side chain. 24-EBL has R orientation, while 28-HBL has orientation of the side chain at C-24.

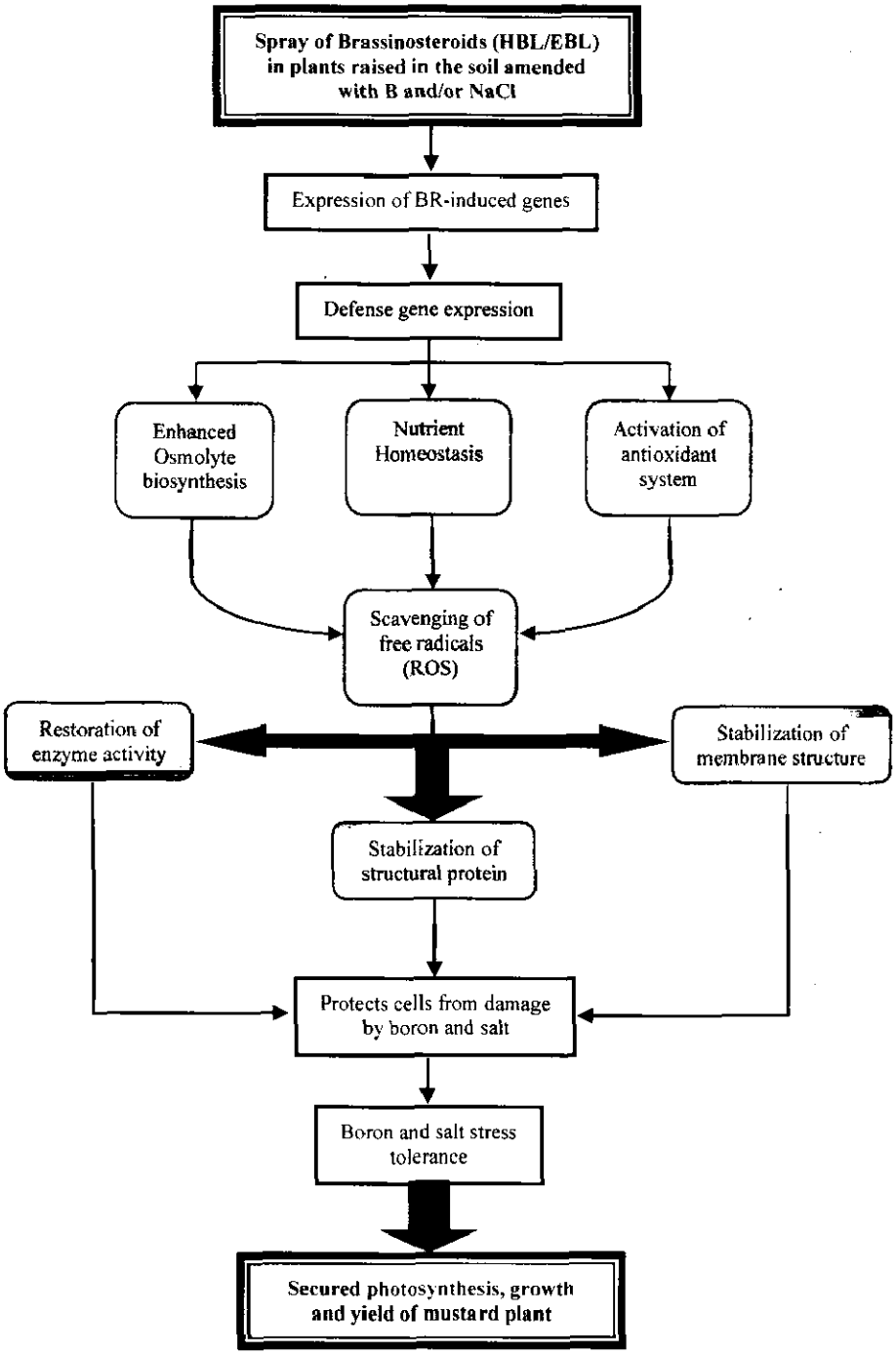


Plate VII: The possible mechanisms involved in the B and salt stress tolerance through the use of brassinosteroids

Chapter 6

SUMMARY

SUMMARY

This thesis comprises of the following five chapters.

- **Chapter 1** introduces the significance of the problem entitled, "Effects of brassinosteroids on boron and salt induced changes in Indian mustard (*Brassica juncea* L.).
- **Chapter 2** reviews the available literature related with the above problem, in terms of growth, metabolism, and yield characteristics of the plants.
- **Chapter 3** elaborates the details of the materials and methods employed in conducting the experiments and, physical and chemical analysis of the biological material.
- **Chapter 4** comprises of tabulated data, recorded during the aforesaid study, and a brief description of the observations.
- **Chapter 5** deals with the possible explanations for the observations, in the light of the earlier findings.

The summary of the observations, recorded in each of the six experiments is given below:

Experiment 1

This experiment was conducted with an objective to study the effects of soil amended six doses of boron (B) on *Brassica juncea* (L.) Czern & Coss, var. Varuna and Chapka Rohini. The healthy looking seeds of both varieties were surface sterilized with 0.01% mercuric chloride solution for 5 min, followed by washing with double distilled water (DDW), at least thrice, to remove the traces of mercuric chloride adhered to the seed surface. The seeds of both the varieties were then sown in earthen pots (25×25 cm) filled with sandy loam soil and farmyard manure, mixed in a ratio of 6:1. The six concentrations (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of boron (B) were amended into the soil, prior to seed sowing. Each treatment was represented by five pots with three plants per pot. The pots were arranged in a simple randomized block design, in the net house of Department of Botany, Aligarh Muslim University, Aligarh. Selected number of plants, at 45 and 60 days after sowing (DAS) were assessed for growth characteristics, chlorophyll content (SPAD level), electrolyte leakage, net photosynthetic rate and its related attributes [stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E)], maximum quantum yield of PSII (Fv/Fm), activity of carbonic anhydrase and nitrate reductase and various

antioxidant enzymes such as catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) along with proline content. The remaining plants were allowed to grow up to maturity and were harvested approximately 120 DAS, to study the yield characteristics. B (20 mg kg^{-1}) generated minimum toxicity whereas, 60 mg kg^{-1} triggered maximum toxicity. All the aforesaid parameters, except electrolyte leakage, antioxidant enzymes and proline content significantly decreased as the level of the B increased in the soil. Moreover, the plants of the two varieties showed significantly different response to graded concentrations of B. The variety Varuna exhibited lesser damage than variety Chapka Rohini, in response to different concentrations of B. Varuna possessed higher activity of antioxidant enzymes at all the levels of B than Chapka Rohini. At harvest, all yield attributes i.e. number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant exhibited a significant reduction in response to B stress.

Experiment 2

This experiment was carried out to study the impact of lower concentration (2.8 dSm^{-1}) of NaCl in the presence or absence of B (20 or 60 mg kg^{-1}) in the two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. All the agricultural practices remained the same as in Experiment 1. The salt (2.8 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) were mixed in the soil prior to seed sowing. The parameters and the pattern of assessment were same as in Experiment 1. The presence of NaCl alone or in combination with B in the soil significantly decreased the growth characteristics, SPAD chlorophyll level, photosynthetic attributes, maximum quantum yield of PSII and activity of nitrate reductase and carbonic anhydrase enzymes whereas, the electrolyte leakage, proline content and activity of antioxidant enzymes (CAT, POX and SOD) increased in both the varieties. Out of the various treatments tested, NaCl (2.8 dSm^{-1}) + B (60 mg kg^{-1}) was most toxic and generated maximum damage in both the varieties, at both the stages (45 and 60 DAS) of growth. The variety Chapka Rohini was more susceptible to NaCl and/or B stress than Varuna.

Experiment 3

This experiment was conducted with an aim to study the impact of moderate concentration (4.2 dSm^{-1}) of NaCl in the presence or absence of B (20 or 60 mg kg^{-1}) in two varieties i.e. Varuna and Chapka Rohini of *Brassica juncea* (L.) Czern & Coss. All the agricultural practices and parameters studied remained the same as in Experiment 1. Salt treatment in the form of NaCl (4.2 dSm^{-1}) and B treatments (20 or

60 mg kg^{-1}) in the form of boric acid was administered through soil prior to seed sowing. The plants grown in the soil amended with NaCl and/or B significantly decreased the values of all growth biomarkers, SPAD chlorophyll content, net photosynthetic rate and its related attributes, maximum quantum yield of PSII (Fv/Fm) and the activity of nitrate reductase and carbonic anhydrase enzymes except the electrolyte leakage, activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) along with proline content in both the varieties. The variety Varuna was less responsive to NaCl and/or B stress than Chapka Rohini. Out of the various treatments tested, NaCl (4.2 dSm^{-1}) + B (60 mg kg^{-1}) was most deleterious in both the varieties, at 45 and 60 day stage of growth. Moreover, the damage caused by NaCl and/or B was higher at 45 day stage of growth compared to 60 day stage of growth.

Experiment 4

This experiment was performed with an objective to study the response to higher concentration (5.6 dSm^{-1}) of NaCl alone or in the presence of B (20 or 60 mg kg^{-1}) in the two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. All the agricultural practices remained the same as in Experiment 1. The treatment of NaCl (5.6 dSm^{-1}) and/or B (20 or 60 mg kg^{-1}) was given to the soil prior to seed sowing. The parameters and the pattern of assessment were same as in Experiment 1. The presence of NaCl alone or in combination of B in the soil decreased significantly the values of growth characteristics, SPAD chlorophyll level, photosynthetic attributes, maximum quantum yield of PSII (Fv/Fm) and activity of carbonic anhydrase and nitrate reductase enzymes whereas, the electrolyte leakage, proline content and activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) increased in both the varieties. However, among all treatments tested, NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}) triggered maximum toxicity in both the varieties, at both 45 and 60 day stage of growth. The variety Chapka Rohini was more susceptible to NaCl and/or B stress than Varuna.

Experiment 5

This experiment was laid down with an aim to explicate the role of two BR analogues i.e. 28-homobrassinolide (HBL) or 24-epibrassinolide (EBL) in the presence of B-induced changes in *Brassica juncea* (L.) Czern & Coss var. Varuna and Chapka Rohini. All the agricultural practices remained the same as in Experiment 1. These two varieties of *B. juncea* were sown in the soil amended with B (20 or 60 mg kg^{-1})

and the foliage of resulting plants was sprayed with deionized water (control), or BRs (10^{-8} M of HBL/EBL) at 44 days after sowing. The plants were subjected to analysis at 45 and 60 DAS for the parameters studied in Experiment 1. The remaining plants were allowed to grow to maturity and were harvested (120 DAS) to study the yield characteristics. The presence of B in the soil caused a significant reduction in growth, chlorophyll content (SPAD level), photosynthetic attributes, quantum yield of PSII, and activity of carbonic anhydrase and nitrate reductase and yield characteristics in a concentration dependent manner but electrolyte leakage, activity of antioxidant enzymes (CAT, POX and SOD) along with proline content increased with the level of B in both the varieties. The variety Chapka Rohini was more susceptible to B stress than Varuna. Moreover, the foliar spray of BRs analogues (HBL/EBL) alone or as a follow-up treatment to B stressed plants improved the values of most of the parameters and completely alleviated the adverse effects generated by the two concentrations (20 or 60 mg kg^{-1}) of B which were prominent in Varuna. The BRs application had an additive effect in increasing the antioxidant enzymes activity and proline content in the stressed plants. Moreover, application of either of the BR analogues improved the yield of the plants both under stress free or stress conditions. Out of the two active analogues of BR tested, 24-epibrassinolide (EBL) was excelled in its effect over HBL.

Experiment 6

This experiment was designed to explicate the impact of 24-epibrassinolide (EBL), under combined stress of the salt and B in two varieties (Varuna and Chapka Rohini) of *Brassica juncea* (L.) Czern & Coss. All the agricultural practices and the parameters studied remained the same as in Experiment 1. NaCl and B [NaCl (2.8 dsm^{-1}) + B (60 mg kg^{-1}), NaCl (4.2 dsm^{-1}) + B (60 mg kg^{-1}) or NaCl (5.6 dsm^{-1}) + B (60 mg kg^{-1})] treatments were given in the soil before sowing. Foliage of 44 day old plants was sprayed with DDW/aqueous solution (10^{-8} M) of EBL. The characteristic studies and their assessment pattern was the same as mentioned in experiment 1. Out of the different combinations of the NaCl and B tested, NaCl (5.6 dsm^{-1}) + B (60 mg kg^{-1}) triggered maximum toxicity (stress) and caused a significant decline in the values of most of the parameters, except those of electrolyte leakage, proline content and activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) that increased in both the varieties. The variety Chapka Rohini was more susceptible to stress than Varuna. However, foliar spray of EBL alone or in the presence of

NaCl + B improved the values of the aforesaid parameters more effectively in Varuna than Chapka Rohini. Moreover, EBL caused a further stimulation in the activity of antioxidant enzymes and proline content which were already higher under the stress conditions generated by NaCl and B. The application of EBL completely restored the values of almost all above parameters against NaCl and B stress more promisingly against NaCl (2.8 or 4.2 dSm^{-1}) + B (60 mg kg^{-1}) and partially that of NaCl (5.6 dSm^{-1}) + B (60 mg kg^{-1}). Varuna possessed higher values than Chapka Rohini and expressed better response to the application of EBL in the alleviation of stress of NaCl with B.

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APPENDIX

APPENDIX

1 Preparation of reagents for the estimation of carbonic anhydrase activity

1.1 ***Cysteine hydrochloride solution (0.2 M)***

48 g of cysteine hydrochloride was dissolved in sufficient DDW and final volume was made up to 1000 cm^3, by using DDW.

1.2 ***Sodium Phosphate buffer (pH 6.8)***

27.80 g of NaH_2PO_4 and 53.65 g of Na_2HPO_4 were dissolved separately in sufficient DDW and final volume was made up to 1000 cm^3. 51 cm^3 of NaH_2PO_4 and 49 cm^3 of Na_2HPO_4 were then mixed to get the required solution.

1.3 ***Alkaline sodium bicarbonate solution***

16.80 g of sodium bicarbonate ($NaHCO_3$) was dissolved in aqueous 0.2 M NaOH solution [0.8 g NaOH (1000 cm^3)$^{-1}$] and final volume was made up to 1000 cm^3, by using DDW.

1.4 ***0.002% bromothymol blue***

0.002 g of bromothymol blue was dissolved in sufficient DDW and final volume was made up to 1000 cm^3 by using DDW.

1.5 ***0.5 N HCl***

4.3 cm^3 of pure HCl was pipetted in sufficient DDW and final volume was made up to 1000 cm^3, by using DDW.

1.6 ***Methyl red indicator***

5 mg of methyl red was dissolved in sufficient ethanol and final volume was made to 100 cm^3, using ethanol.

2 Preparation of reagents for the estimation of nitrate reductase activity

2.1 ***0.1 M Phosphate buffer (pH 7.4)***

27.20 g of KH_2PO_4 and 45.63 g of $K_2HPO_4.7H_2O$ were dissolved separately in 1000 cm^3 of DDW. The above solutions of KH_2PO_4 and $K_2HPO_4.7H_2O$ were then mixed in the ratio of 16:84 to get the solution of required concentration.

2.2 ***0.2 M KNO_3***

20.20 g of KNO_3 was dissolved in sufficient DDW and final volume was made up to 1000 cm^3, using DDW.

2.3 ***5% Isopropanol***

5 cm^3 of isopropanol was pipetted into sufficient DDW and final volume was made up to 100 cm^3, using DDW.

4 *1% Sulphanilamide*

1 g of sulphanilamide was dissolved in 100 cm^3 of 3 N HCl which was prepared by dissolving 25.86 cm^3 of HCl in sufficient DDW and final volume was maintained to 100 cm^3, by using DDW.

5 *0.02% N-1-Naphthylethylenediamine dihydrochloride (NED-HCl)*

20 mg of NED-HCl was dissolved in sufficient DDW and final volume was made up to 100 cm^3, by using DDW.

Preparation of reagents for catalase estimation

1 *Phospahte buffer (0.1 M) for pH 6.8*

3.54 g of Na_2HPO_4 was dissolved in 100 cm^3 of DDW and 3.72 g of NaH_2PO_4 was added to 100 cm^3 of DDW. Then 12.3 cm^3 of Na_2HPO_4 was added to 87.7 cm^3 of NaH_2PO_4 to get the solution of required concentration and pH.

2 *H_2O_2 (0.1M)*

0.34 cm^3 of H_2O_2 was added to 100 cm^3 of DDW.

3 *Sulphuric acid (2%)*

2 cm^3 of H_2SO_4 was added to 98 cm^3 of DDW.

4 *0.1 N Potassium permanganate*

This was made by dissolving 0.162 g of $KMnO_4$ in 500 cm^3 of DDW.

Preparation of reagents for peroxidase estimation

1 *Pyrogallol phosphate buffer*

It was prepared by mixing 25 cm^3 of pyrogallol in 75 cm^3 phosphate buffer (pH 6).

Preparation of reagents for superoxide dismutase

1 *Phosphate buffer (50 mM) for pH 7.8*

It was prepared by mixing 1.78 g Na_2HPO_4 and 1.56 g of NaH_2PO_4 in 100 cm^3 of DDW separately. 91.5 cm^3 of Na_2HPO_4 with 8.5 cm^3 of NaH_2PO_4 were mixed to get phosphate buffer of pH 7.8.

2 *Methionine (13 mM)*

It was prepared by dissolving 0.193 g of methionine in 100 cm^3 of DDW.

3 *Nitrobluetetrazolium (NBT) (75 µM)*

6.13 mg of NBT was dissolved in 100 cm^3 of DDW.

4 *Riboflavin (2 µM)*

0.0753 mg of riboflavin was dissolved in 100 cm^3 of DDW.

5.5 ***EDTA (0.1 M)***

2.92 g EDTA was dissolved in 100 cm^3 of DDW.

6 Preparation of reagents for proline estimation

6.1 ***Sulfosalicylic acid (3%)***

3 g of sulfosalicylic acid was dissolved in sufficient DDW and final volume was maintained to100 cm^3, by using DDW.

6.2 ***Acid ninhydrin solution***

1.25 g of ninhydrin was dissolved in a mixture of warm, 30 cm^3 of glacial acetic acid and 6M phosphoric acid (pH 1.0) with agitation till it got dissolved. It was stored at 4°C and used within 24 h.

The 6 M phosphoric acid was prepared by mixing 11.8 cm^3 of phosphoric acid with 8.2 cm^3 of DDW.

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RESEARCH ARTICLE

Boron induced modulation in growth, photosynthesis and antioxidant system in two varieties of *Brassica juncea*

Priyanka Varshney, Qazi Fariduddin*, and Mohammad Yusuf
Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh, India

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Corresponding Author

Qazi Fariduddin

Abstract

Boron toxicity is an environmental constraint that limits crop productivity worldwide. The aim of the present study was to explore the boron-induced modulation in growth, photosynthesis, antioxidant system under varied levels of boron in two varieties of *Brassica juncea* L. Czern & Coss var. Varuna and Chapka rohini. The surface-sterilized seeds of these varieties were sown in soil amended with different levels (0, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) of boron and were sampled at 45 d stage of growth. The boron treatments (20, 30, 40, 50 or 60 mg kg^{-1}) significantly decreased growth, net photosynthetic rate and its related attributes, chlorophyll fluorescence, SPAD value of chlorophyll, and leaf carbonic anhydrase and nitrate reductase activities whereas, the proline content and the level of various antioxidant enzymes (catalase, peroxidase and superoxide dismutase) increased in both the varieties. Out of the graded concentrations of boron, 20 mg kg^{-1} was least toxic and 60 mg kg^{-1} B generated maximum toxicity. However, the application of 10 mg kg^{-1} B did not generate any significant effect in almost all the parameters. Furthermore, the variety Varuna was found more tolerant than Chapka rohini to the boron stress and possessed higher values for growth, photosynthetic attributes and antioxidant enzymes. The variation in the responses of these two varieties to boron toxicity is attributed to their differential photosynthetic traits, SPAD chlorophyll value and antioxidant capacity, which could be used as potential markers for screening mustard plants for boron tolerance.

INTRODUCTION

Boron (B) is an essential micronutrient required for the normal growth of higher plants and is absorbed by plants from soil solution in the form of boric acid (Dordas et al., 2000). There is a narrow window between deficiency and toxicity in soil-plant systems however, the risk of inducing toxicity should not be ignored (Herrera-Rodriguez et al., 2010; Siddiqui et al., 2013). The content of B varies in the soil as some soils contain insufficient B to support normal plant growth, while others contain excess of B, which causes toxicity in plants (Esim et al., 2012). It has long been known that the optimum B level for one species could be either toxic or insufficient for other species (Blevins and Lukaszewski, 1998). In soils deficiency may take place below 0.5 mg B kg^{-1} while soil containing more than 5 mg B kg^{-1} could cause toxicity symptoms in most of the crops (Gupta, 2007).

Boron toxicity is an important micronutrient disorder affecting the productivity of many cultivated crops across the world, including oilseed crops i.e., Brassicas (Campbell et al., 1998). The most obvious symptoms of toxicity are leaf burns and chlorotic and necrotic patches, often visible at the margins and tips of older leaves. These symptoms reflect the tissue distribution of boron in most species, with accumulation at the ends of the transpiration stream (Nable et al., 1990). Plant growth and seed yield are typically reduced when boron toxicity is present (Yau and Saxena, 1997). In addition, B toxicity causes physiological and morphological defects in plants such as

decreased shoot and root growth (Lovatt and Bates, 1984; Nable et al., 1997), inhibition of photosynthesis and lower stomatal conductance (Lovatt and Bates, 1984), decreased proton extrusion from roots (Roldan et al., 1992), decreased root cell division (Liu et al., 2000; Choi et al., 2007), deposition of lignin and suberin in the roots (Ghanati et al., 2002), increased membrane permeability, lipids peroxidation and hydrogen peroxide (H_2O_2) content, and changed the activities of antioxidant enzymes (Herrera-Rodriguez et al., 2010; Esim et al., 2012).

Like other abiotic stresses (drought, salinity, cold, heat and heavy metals), excess B lead to the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$) radicals, hydrogen peroxide, and free singlet oxygen (Siddiqui et al., 2013; Archana and Pandey 2015; Landi et al. 2014). These over-accumulated ROS trigger detrimental effects on cellular processes, including oxidative damage to nucleic acids, proteins, lipids, and resulting in the alteration in antioxidative enzymes activities (Molassiotis et al., 2006; Tombuloglu et al., 2012). To prevent themselves from the harmful effects of these reactive molecules, plant have evolved an effective scavenging system of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase (Apel and Hirt, 2004). Among them, superoxide dismutase as a major scavenger decomposes superoxide ion ($O_2^{\bullet-}$) to H_2O_2 and molecular oxygen that are further detoxified to O_2 and H_2O by catalase and/or peroxidase via ascorbate-glutathione pathway (Karabal et al., 2003). It was observed that high supply of B invoked the formation of ROS which induced oxidative damage by lipid peroxidation and accumulation of hydrogen peroxide in leaves (Karabal et al., 2003; Molassiotis et al., 2006). Cervilla et al. (2012) also reported oxidative damage and altered antioxidative enzyme activity in tomato leaves under B toxicity, indicating a crucial role of antioxidant system in conferring tolerance to B stress in plants.

The present study was carried out to examine the changes in growth, photosynthetic characteristics, chlorophyll pigments, chlorophyll fluorescence, and level of non-enzymatic and enzymatic antioxidants in two varieties i.e., Varuna and Chapka rohini of mustard (*Brassica juncea* L.) to the different concentrations of B.

MATERIALS AND METHODS

Plant material and experimental design

The authentic and healthy seeds of *Brassica juncea* L. var. Varuna and Chapka rohini obtained from National Seed Corporation Ltd. (New Delhi, India) were surface sterilized with 1% sodium hypochlorite solution, followed by rinsing with deionized water at least thrice. These surface-sterilized seeds of two varieties were sown in earthen pots (20 cm in diameter, 20 cm in depth) filled with sandy loam soil and farmyard manure (mixed to the ratio of 6:1) and lined in a net house, where the average day/night temperature, relative humidity and photoperiod were 25°C/20, 6±3% and 12 h, respectively. Before sowing seeds, the different levels of B (0, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) were given through the soil in the form of boric acid (H_3BO_3). Thinning was done on the 7th day after sowing (DAS), leaving three plants per pot. Each treatment was represented by five pots. Irrigation was done with tap water as and when required. The plants were up-rooted at 45 DAS to assess the growth, photosynthetic and biochemical parameters. The remaining plants were allowed to grow up to maturity and were harvested at 120 DAS to study the yield characteristics.

Plant growth analysis

The plants were uprooted and washed under running tap water to dislodge the soil particles to analyze the several growth parameters. The length of the shoot and root was measured on a meter scale. The shoots and roots were weighed separately to record their fresh mass and placed in an oven run at 80°C for 72 h. The samples were weighed again to obtain their respective dry mass. The leaf area was measured by leaf area meter (ADC Bioscientific, Hoddesdon, Herts, UK).

Determination of SPAD value of chlorophyll and photosynthetic parameters

The chlorophyll content in the leaves was measured with the help of a SPAD chlorophyll meter (Konica Minolta Sensing Inc., Japan). The rate of photosynthesis and its related parameters (photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate, were measured by using a portable photosynthesis system (LI-COR 6400; LI-COR Lincoln, NE, USA). Where, the air temperature, relative humidity, CO_2 concentration and photosynthetic photon flux density (PPFD) were maintained at 25°C, 85%, 600 µmol mol^{-1} and 800 µmol mol^{-2} s^{-1}, respectively. The measurements were made in the uppermost fully expanded leaves between, 11:00 and 13:00 hours.

Analysis of chlorophyll fluorescence i.e. maximum quantum yield of photosystem II (Fv/Fm)

Chlorophyll fluorescence i.e., maximum quantum yield of photosystem II (Fv/Fm) was measured by using a leaf chamber fluorometer (LI-COR 6400-40, LI-COR Lincoln, NE, USA). All the measurements were carried out at a PPFD of 1,500 µmol m^{-2} s^{-1} with a constant airflow rate of 500 µmol s^{-1}. The sampled leaf was dark adapted for 30 min, prior to the measurement of Fv/Fm.

Determination of nitrate reductase and carbonic anhydrase activity

Nitrate reductase activity was measured following the method laid down by Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5), KNO_3 and

isopropanol which were incubated at 30°C for 2 h. After incubation, sulfanilamide and N-1-naphthylethylenediamine hydrochlorides solutions were added. The absorbance was read at 540 nm on a spectrophotometer (Spectronic 20D; Milton Roy, USA). Carbonic anhydrase activity was determined using the procedure described by Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces in cystein hydrochloride solution. These samples were blotted and transferred to the test tubes followed by the addition of phosphate buffer (pH 6.8), 0.2 M $NaHCO_3$, bromothymol blue, and the methyl red indicator, at the last. This reaction mixture was titrated against 0.05 N HCl. The activity of the enzyme was expressed on a fresh mass basis.

Antioxidant enzyme activity

For the assay of antioxidant enzymes, the leaf tissue (0.5 g) was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 g for 10 min at 4°C and the supernatant was used as source of enzymes catalase, peroxidase and superoxide dismutase. Catalase and peroxidase were assayed following the method described by Chance and Maehly (1956). Catalase was estimated by titrating the reaction mixture consisting of phosphate buffer (pH 6.8), 0.1 M H_2O_2, enzyme extract and 2% H_2SO_4 against 0.1N $KMnO_4$ solution. The reaction mixture for peroxidase consisted of pyragallol, phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract. Change in absorbance due to catalytic conversion of pyragallol to perpurogallin was noted at an interval of 20 s for 2 min, at 420 nm on a spectrophotometer. A control set was prepared by using deionized water instead of enzyme extract. The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Beauchamp and Fridovich (1971). The reaction mixture consisted 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0-50 µL enzyme extract and were placed under 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light. Non-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm and the SOD activity was expressed as unit g^{-1} fresh mass. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photo-reduction.

Determination of proline content

The proline content in fresh leaf was determined by adopting the method of Bates et al., (1973). The samples were extracted in sulphosalicylic acid. To the extract, an equal volume of glacial acetic acid and ninhydrin solutions was added. The sample was heated at 100°C, to which 5 mL of toluene was added after cooling in ice bath. The absorbance of toluene layer was read at 528 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA).

Statistical analysis

Data were statistically analyzed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Standard error was calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference (LSD) between treatment means with the level of significance at $P \leq 0.05$.

RESULTS

Growth biomarkers

The length, fresh and dry mass of the shoot and root, and leaf area of both the varieties showed a marked decrease on being subjected to different levels of B (Fig. 1 and 2A). Out of the different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B, 10 mg kg^{-1} did not generate significant effect whereas, 20 mg kg^{-1} proved least toxic. However, the highest concentration of B (60 mg kg^{-1}) generated severe damage and caused maximum per cent decrease in the shoot length by 24% and 39%, root length by 31% and 44%, shoot fresh mass by 26% and 39%, root fresh mass by 37% and 51%, shoot dry mass by 30% and 39%, root dry mass by 36% and 43% and leaf area by 28% and 41% in Varuna and Chapka rohini respectively, compared with their respective controls. The damage was more prominent in Chapka rohini than in Varuna.

SPAD value of chlorophyll

Maximum chlorophyll content was recorded in unstressed (control) plants where the variety Varuna possessed higher values for chlorophyll content than Chapka rohini (Fig. 2B). Out of different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B, 10 mg kg^{-1} did not generate significant decline but above this there was observed significant decline in chlorophyll content. Moreover, the highest concentration (60 mg kg^{-1}) of B caused maximum decrease by 26% and 38% in Varuna and Chapka rohini respectively, compared to their controls.

Photosynthetic parameters

The plants raised from the seeds sown in the soil fed with different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B except 10 mg kg^{-1} showed significant decrease in the net photosynthetic rate (P_N) and its related attributes [stomatal conductance (g_s), internal CO_2 concentration (Ci) and transpiration rate (E)] in Varuna and Chapka rohini (Fig. 2C-F). The decrease was proportionate to the concentrations of B. The highest concentration (60 mg kg^{-1}) of B decreased P_N, g_s, Ci and E by 31%, 35%, 25%, and 22% in Varuna and 44%, 50%, 41% and 36% in Chapka rohini

respectively, when compared to their control plants. Moreover, the Chapka rohini was more sensitive to B stress than Varuna.

Maximum quantum yield of photosystem II (Fv/Fm)

Fig. 3A shows that the presence of different concentrations (20, 30 40, 50 or 60 mg kg^{-1}) of B in the soil decreased the maximum quantum yield of photosystem II (Fv/Fm) in both the varieties. However, the highest concentration of B (60 mg kg^{-1}) proved most deleterious and decreased the Fv/Fm by 26% and 38% in Varuna and in Chapka rohini respectively, compared to their controls. Moreover, 10 mg kg^{-1} B did not generate significant impact in Fv/Fm. The order of toxicity of B was 20<30<40<50<60 mg kg^{-1}. The variety Varuna possessed higher values for Fv/Fm than Chapka rohini.

Carbonic anhydrase and nitrate reductase activity

The plants raised in the presence of different levels (20, 30, 40, 50 or 60 mg kg^{-1}) of B had lower activity of carbonic anhydrase and nitrate reductase enzymes compared with unstressed (control) plants in concentration dependent manner (Fig. 3B and C). Therefore, 60 mg kg^{-1} generated maximum toxicity in both carbonic anhydrase (18% and 30% lower than control) and nitrate reductase (28% and 36% lower than control) in Varuna and Chapka rohini respectively, compared to the respective controls. Moreover, 10 mg kg^{-1} B did not induce significant effect in carbonic anhydrase and nitrate reductase activity. The loss in the activity of these enzymes was more prominent in Chapka rohini than Varuna.

Antioxidant enzymes

Unlike the other parameters, the activity of antioxidant enzymes catalase, peroxidase and superoxide dismutase showed completely different response (Fig. 3D-F). The data revealed that the antioxidant enzyme activity increased in response to the concentrations (20, 30, 40, 50 or 60 mg kg^{-1}) of B in the soil in both the varieties. The plants raised in the soil amended with the highest B level (60 mg kg^{-1}) possessed maximum values for antioxidant enzymes in both the varieties. The values for catalase, peroxidase and superoxide dismutase activity increased by 40%, 44% and 54% in Varuna and 25%, 30% and 39% in Chapka rohini respectively, compared to their respective control plants.

Proline content

As evident from the Fig. 4A, the leaf proline content was higher in the plants that were raised in the presence of excess B in the soil. The values increased with an increase in the concentration of the B, whereas 10 mg kg^{-1} did not generate any significant increase in proline content. Maximum values were found in the plants which were fed with 60 mg kg^{-1} of B through the soil in both the varieties and the increase was 69% and 51% in Varuna and Chapka rohini respectively, over the respective controls.

Yield attributes

Yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) were significantly affected and exhibited a linear decrease in their values in response to the different concentrations of B (20, 30 40, 50 or 60 mg kg^{-1}) present in the soil in both the varieties, at harvest (Fig. 5). The maximum reduction in the values of all yield attributes was noticed at 60 mg kg^{-1} of B and decreased the number of pods per plant, number of seeds per pod, 100 seed mass and seed yield by 28%, 19%, 15% and 25% in Varuna and 34%, 27%, 24% and 31% in Chapka rohini at respectively, compared to their control plants. Furthermore, there was no significant reduction in all yield parameters at 10 mg kg^{-1}. The variety Chapka rohini was more prone to the stress than the Varuna.

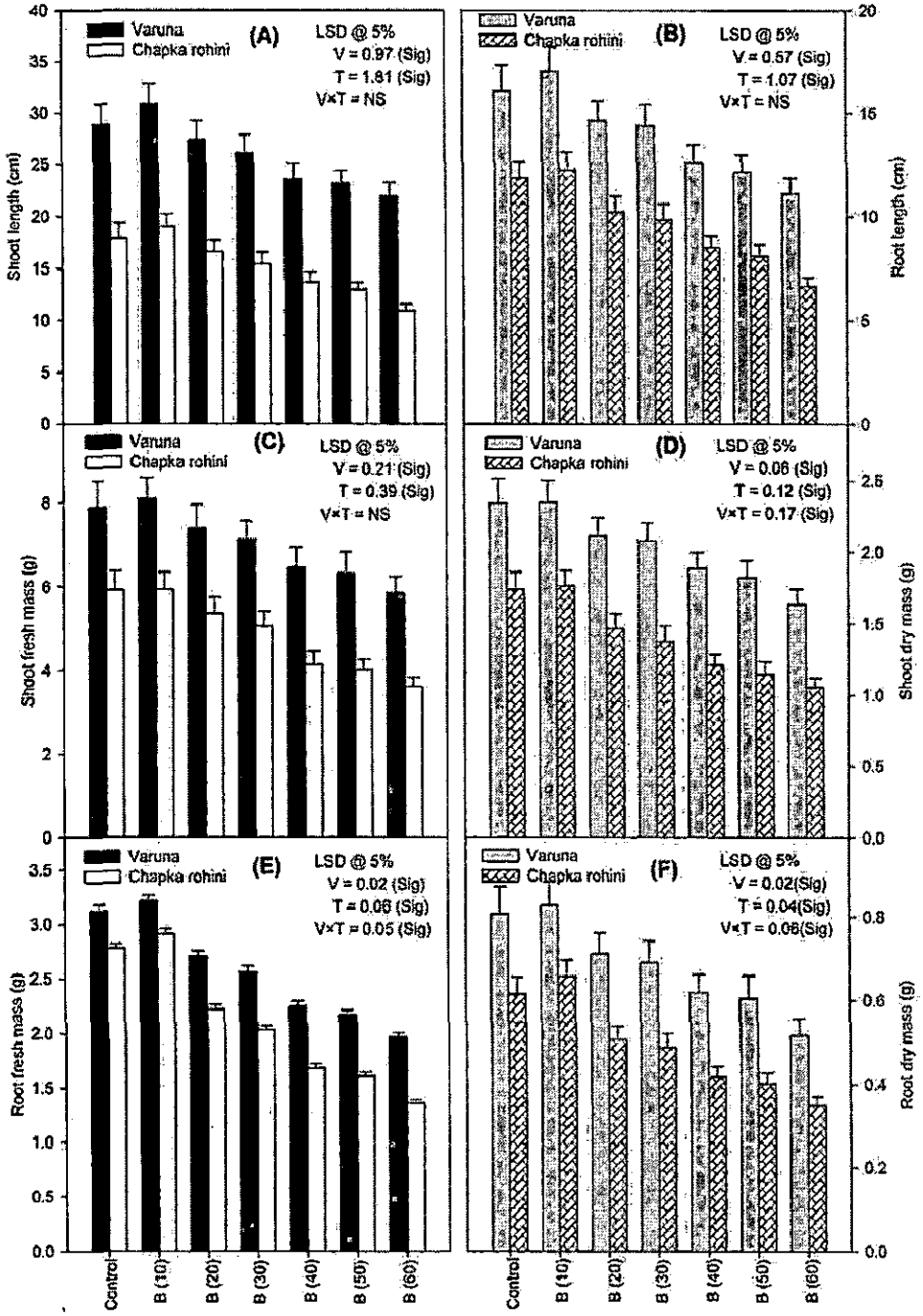

Fig. 1 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) shoot length, (B) root length, (C) shoot fresh mass, (D) shoot dry mass, (E) root fresh mass, (F) root dry mass, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

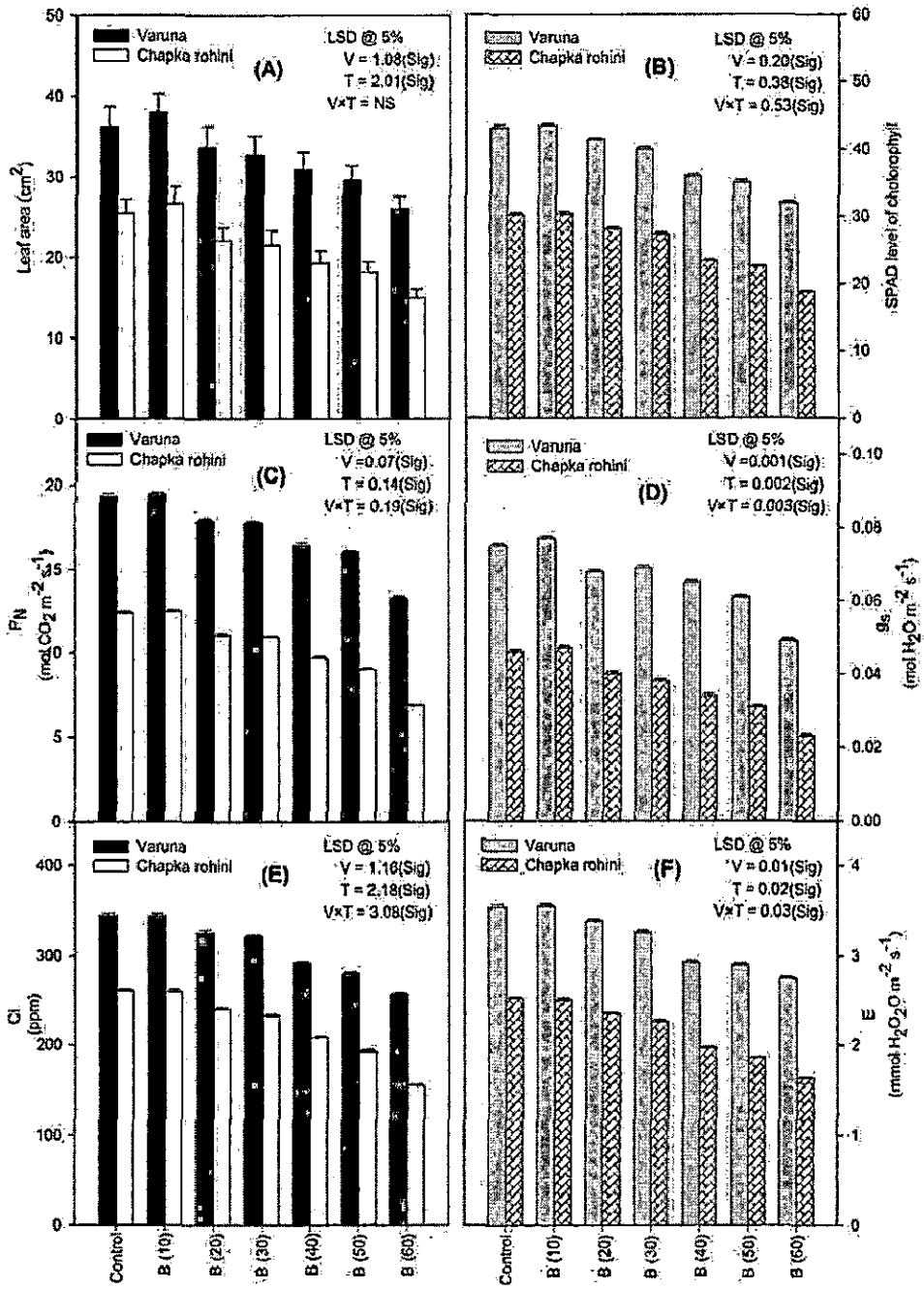

Fig. 2 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) leaf area, (B) SPAD value of chlorophyll, (C) net photosynthetic rate (P_N), (D stomatal conductance (g_s), (E)) internal CO_2 concentration (Ci), (F) transpiration rate (E), in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

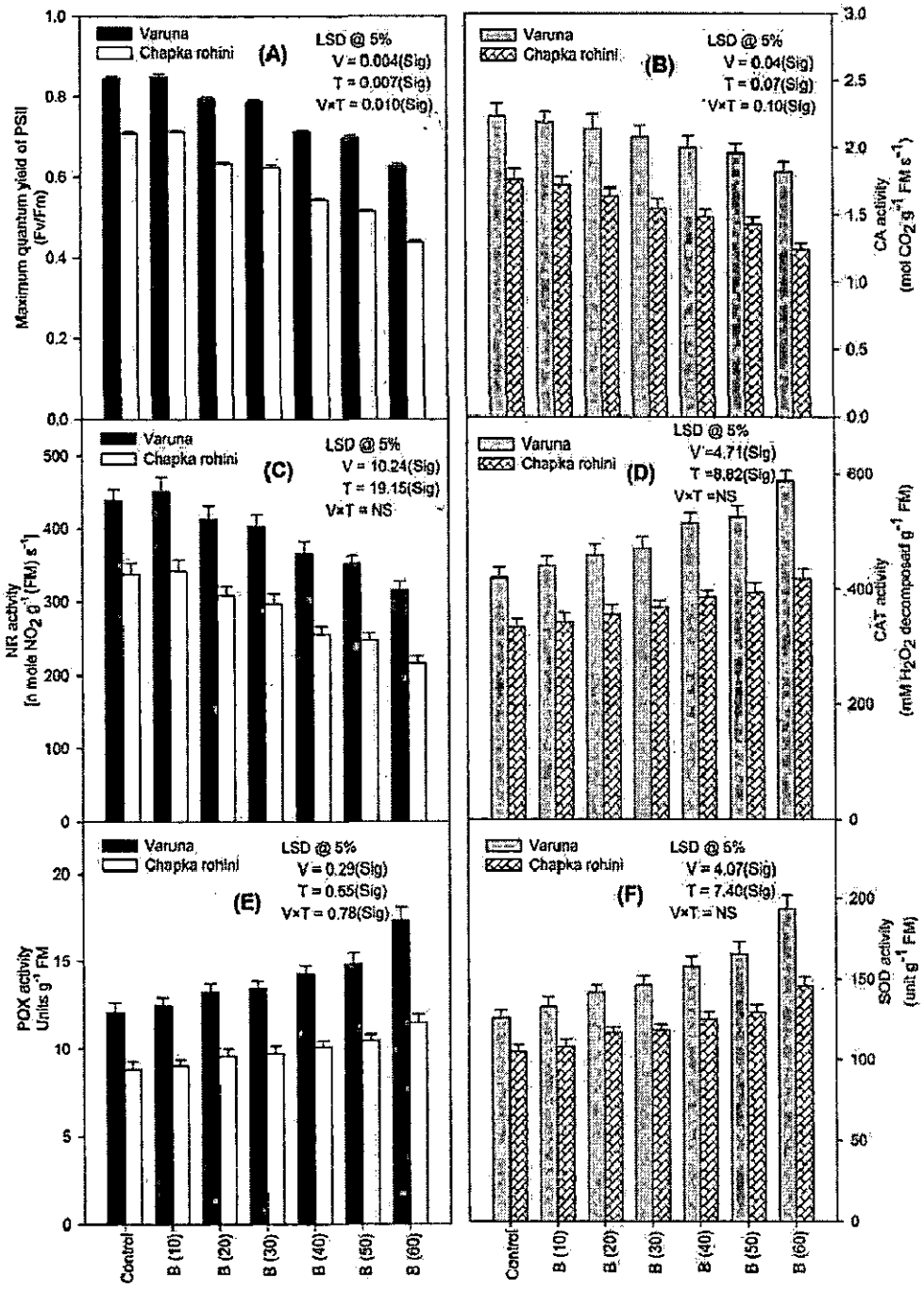

Fig. 3 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) maximum quantum yield of photosynthesis (Fv/Fm), (B) carbonic anhydrase (CA) activity, (C) nitrate reductase (NR) activity, (D) catalase (CAT) activity, (E) peroxidase (POX) activity, (F) superoxide dismutase (SOD) activity, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

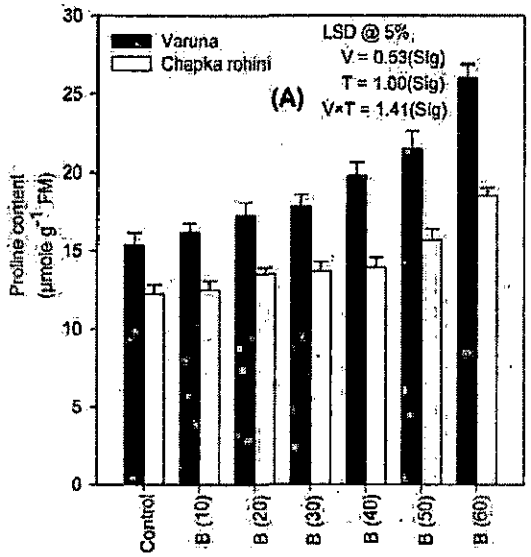

Fig. 4 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) leaf proline content, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

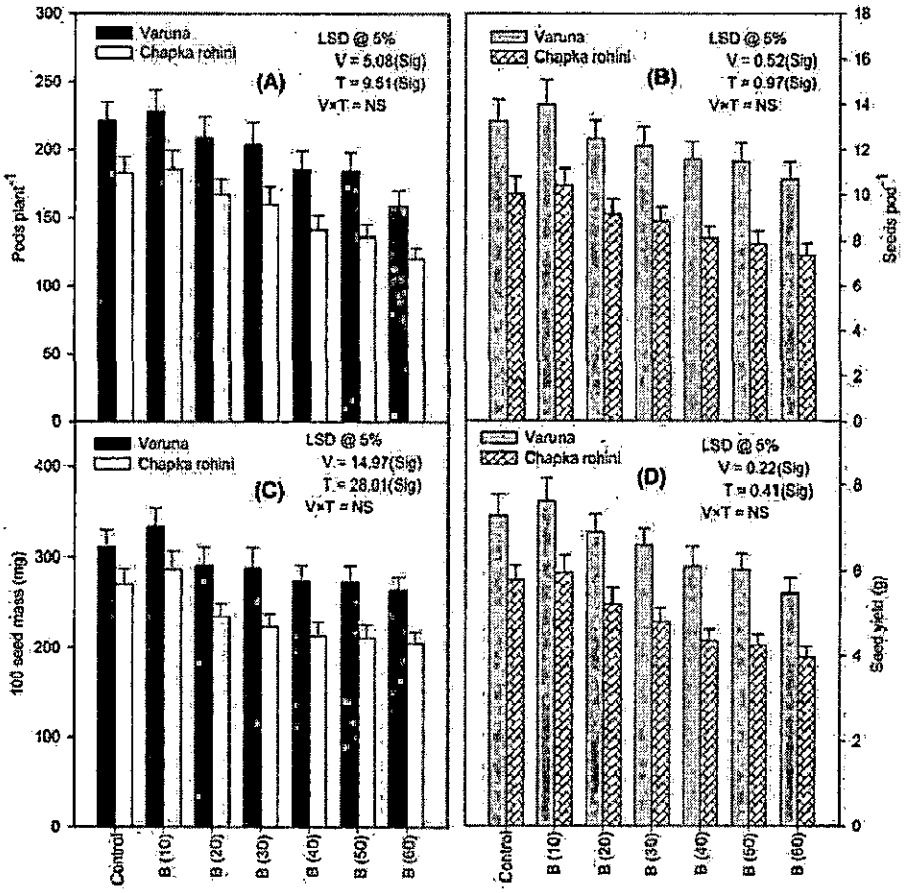

Fig. 5 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) pods $plant^{-1}$, (B) seeds pod^{-1}, (C) 100 seed mass, (D) seed yield, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

DISCUSSION

All the growth parameters (fresh and dry mass of roots and shoots, their lengths and the leaf area) was not significantly reduced in mustard plants grown in the soil amended with 10 mg kg^{-1} of B but was significantly reduced with 20, 30, 40, 50 or 60 mg kg^{-1} of B on both varieties i.e. Varuna and Chapka rohini (Fig. 1 and 2A). High levels of B suppressed plant growth which might be due to the reduction in cell division and elongation (Lovatt and Bates, 1984; Nable et al., 1997) because these processes are responsible in the retardation of normal biosynthetic activities or energy transduction, protein synthesis, and inhibition of many cellular processes (Reid et al., 2004) and altered activities of antioxidant enzymes (Karabal et al., 2003; Keles et al., 2004) which will naturally impair plant growth and finally the yield (Fig. 5). These results are in conformity with tomato (Cervilla et al., 2009), apple rootstocks (Mouhtaridou et al., 2004), mustard (Javid et al., 2014), wheat (Coskun et al., 2014) and watermelon (Hamurcu et al., 2015). Present study also revealed that the root growth (root length, fresh weight and dry weight) was more affected than the growth of shoots due to B toxicity in both the varieties i.e., Varuna and Chapka rohini (Fig. 1). This might be due to the more accumulation of B in roots that resulted in reduced elongation and lateral root development (Kaur et al., 2006). The inhibition in root elongation may be correlated with a decrease in either cell numbers or cell width (Choi et al., 2007; Ardic et al., 2009). Furthermore, out of the two varieties, Varuna is likely more tolerant as reflected by minimum loss in plant growth. This relative tolerance of genotype of a species to B could possibly be due to differences in their abilities to passively transport of B, probably due to differences in membrane composition affecting transmembrane movement of B. Lipid composition of the plasma membrane could affect the total uptake of B in mutants of *Arabidopsis* (Dordas et al., 2000). Therefore, the improved tolerance of Varuna to B could be related to higher exclusion of B in the roots mediated by reduced permeability of membrane lipids and/or presence of carriers (BOR and NIP) essential for exclusion (Miwa et al., 2007).

The SPAD value of chlorophyll decreased significantly in the B stressed (20, 30, 40, 50 or 60 mg kg^{-1}) leaves of both varieties (Fig. 2B). This inhibition of chlorophyll value might be due to the B induced production of reactive oxygen species (ROS) (Reid et al., 2004; Camacho-Cristobal et al., 2008; Han et al., 2009) which lead to photo-oxidative damages in organic molecules (Papadakis et al., 2004). The observations are further corroborated by the findings in *Vigna radiata* (Hasnain et al., 2011) and *Phaseolus vulgaris* (Nagesh et al., 2012). In other studies, oxidative damage in apple and grape (Gunes et al., 2006) and photooxidation damage to organic molecules in orange plants were induced by B toxicity (Cervilla et al., 2007).Besides this, fig. 2C-F revealed that B induced decrease in net photosynthetic rate (P_N) and related attributes (g_s, Ci, and E) along with chlorophyll fluorescence i.e., maximum quantum yield of photosystem II (Fv/Fm) (Fig. 3A) in a concentration dependent manner. One of the probable reason for the reduction of P_N, g_s, Ci, and E (Fig. 2C-F) is the structural damage of thylakoids, which affects the photosynthetic transport of electrons, as indicated by the reduction of the ratio between variable fluorescence and initial fluorescence (Fv/F_0) (Pereira et al., 2000). The decrease in net photosynthetic rate could be attributed to oxidation of chlorophyll and chloroplastic membranes, which might be exacerbated by the excess B in the soil (Lee, 2006; Ardic et al., 2009). Furthermore, increased levels of B reduced the internal CO_2 concentration, so less of the absorbed photon-energy, captured by the light harvesting system, is expected to be used in the electron transport system, thus decreasing photosynthesis (Aftab et al., 2011; Landi et al., 2012). A significant reduction in CO_2 assimilation due to B toxicity has also been reported in various species such as summer squash (Lovatt and Bates, 1984), kiwi fruits (Sotiropoulos et al., 2002), citrus (Han et al., 2009; Sheng et al., 2010) and pear (Wang et al., 2011). The diminution of maximum quantum yield of photosystem II i.e., chlorophyll fluorescence (Fv/Fm) (Fig. 3F) under B stress due to the molecular O_2 operates as an alternative acceptor for non-utilized electrons and light energy (Velez-Ramirez et al., 2011), resulting thus in the generation of ROS. The ability of ROS to cause photooxidative damages in organic molecules could probably explain the structural damages in the chloroplasts, and the reductions of leaf chlorophyll (Fig. 2B; Han et al., 2009; Chen et al., 2012).

The excess B (10, 20, 30, 40, 50 or 60 mg kg^{-1}), except 10 mg kg^{-1} B caused the inhibition of carbonic anhydrase and nitrate reductase e activity (Fig. 3B-C). The variety, Varuna expressed slight tolerance, compared with Chapka rohini. The possible reason behind this may be that B has ability of metabolic disruption by binding to the ribose moieties of molecules such as ATP, NADH or NADPH (Reid et al., 2004) restricting the uptake of nitrate (Harnandez et al., 1996) and also an inhibition and/or metabolic dysfunction of the enzyme protein (Hopkins, 1995). Boron toxicity caused the inhibition of protein synthesis through the formation of borate esters with ribose (Reid 2007) and also altered the activities of several enzymes, and consequently the plant metabolism (Herrera-Rodrigues et al., 2010).

As a natural course plants exposed to stress produce large quantities of ROS (Schutzendubell and Polle, 2002) that may oxidize proteins, lipids and nucleic acids resulting in abnormalities at the level of cell (Sharma et al., 2010). Boron toxicity also causes an oxidative stress because of the formation of ROS such as superoxides and hydroxy and peroxy radicals as induced in many other ionic stress which can damage metabolic processes, altering membranes through lipid per oxidation, and provoking cell death in the plant (Molassiotis et al., 2006). In order to counteract

these ROS, plants induce the synthesis of antioxidant metabolites (proline, ascorbate, glutathione etc.) and enzymes (peroxidase, superoxide dismutase, catalase etc.) that neutralize the toxic effects of ROS generated through stress. We have found that the mustard plants raised the level of endogenous enzymes such as catalase, peroxidase and superoxide dismutase and the non-enzymatic component such as proline in the presence of B stress (Fig. 3D-F and 4A). Our result corroborate previous reports indicating an increase in catalase and superoxide dismutase activity in response to excess B in barley (Karabal et al., 2003), in tomato (Cervilla et al., 2007) in apple rootstocks (Sotiropoulos et al., 2006; Molassiotis et al., 2006), in chickpea (Ardic et al., 2009) while an increase in peroxidase activity has been reported in chickpea (Ardic et al., 2009). Increased accumulation of hydrogen peroxide in leaves and roots of *Brassica juncea* was accompanied by enhanced activities of catalase, peroxidase, and superoxide dismutase also strengthen our findings (Archana and Pandey, 2015). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater tolerance to oxidative damage (Sudhakar et al., 2001). Moreover, the induction of these antioxidant metabolisms coincided with an elevated rate of proline (Fig. 4A) at highest dose (60 mg kg^{-1}) of B, indicating that excess B induced oxidative damage. Increased proline levels are another common response of plants upon oxidative stress (Karabal et al., 2003). The stimulated proline accumulation in the mustard under the influence of higher levels of the applied B is in agreement with the result obtained in tomato and pepper (Eraslan et al., 2007), in wheat (Metwally et al., 2012). Proline also protects enzymes and membranes against oxidative stress (Agarwal and Pandey, 2004). Therefore, in the present study, Varuna possessed enhanced activities of antioxidants like, catalase, peroxidase, superoxide dismutase and higher proline content than in Chapka rohini, which suggest that antioxidative defense system could be one of the effective components of mechanism of tolerance of mustard plants to B toxicity.

Plants exposed to the varying levels of B (10, 20, 30, 40, 50 or 60 mg kg^{-1}) in the soil showed a reduction in yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) soil (Fig. 5). However, variety Varuna performed better and showed a lesser loss in yield than Chapka rohini could be attributed to improved plant growth (Fig. 1 and 2A). Similar findings were reported by Mirshekari (2012) and Cokkizgin (2013), who observed a restricted, seedling vigour index of *Anethum graveolens* and *Phaseolus vulgaris* at high level of B concentrations, respectively. Under conditions of excess B supply, its concentration in the cytosol may rise and cause metabolic disturbances by formation of complexes with NAD^+ of the ribonucleotide units that form a key part of the RNA structure (Loomis and Durst, 1992). The adverse effects of B on plant metabolic activities are more probably related to chlorosis and necrosis, loss of photosynthetic capacity, leading to their poor growth and seed setting and eventually reduction in plant productivity (Reid, 2007) (Fig. 5). Reduction in yield could be attributed to decrease in assimilates under limited water and nutrient supply to the photosynthetic organs in the presence of excessive trace elements (Hasnain et al., 2011).

CONCLUSION

From present study, it concluded that the presence of boron (20, 30, 40, 50 or 60 mg kg^{-1} B added through soil) significantly retarded plant growth, pace of photosynthesis, and ultimately the seed yield in both the varieties viz., Varuna and Chapka rohini of the *Brassica juncea* L. even though the plants exhibited a higher antioxidant enzyme activity and an accumulation of proline content (the protective mechanism). The variety Chapka rohini was more sensitive to the boron toxicity than Varuna.

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